CABBAGE YELLOWS AND THE RELATION OF TEMPERATURE TO ITS OCCURRENCE

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Introduction

In recent years the diseases of plants caused by fungi belonging to the genus *Fusarium* have assumed greater and greater importance from an economic standpoint. A large amount of work has been done on the descriptions of such diseases on new hosts, and on the taxonomy of the genus *Fusarium,* but there has been comparatively little study of the relations of these fungi to their hosts, especially of the conditions under which members of this genus may become harmful parasites. Therefore, any work which throws light on this point is of value scientifically, first, because the mode of attack and the other relations of the parasitic species of *Fusarium* are all very closely related and very similar in their nature, and second, because of the possibility of throwing light on the problem of immunity or resistance of plants to the attack of parasitic organisms. The latter point is of particular interest, since it will be recalled that, up to the present time, practically the only control of the diseases caused by fungi belonging to this genus, has been by the selection or development of strains of the host resistant to fungous attack.

While assisting in the work of the development of strains of cabbage resistant to yellows in Wisconsin, investigations were undertaken to find the cause of the disease and the relations between host and parasite. During these investigations the relation of temperature to the occurrence of this disease was found to be of utmost importance, and the principal part of the work was accordingly devoted to this side of the problem. Nevertheless, before taking up these observations and experiments in detail, the results of the investigations into the etiology and pathological anatomy of the disease should

be discussed, in order that the physiological relations between host and parasite may be understood more clearly. A brief resumé of the literature on cabbage yellows will show the state of our knowledge of this disease at the time these investigations were taken up.

History of the Disease

The disease was first reported by Smith ('99, '99[a]), as occurring in New York State in 1895. He found the trouble exceedingly severe, threatening "to put an end to the successful growing of cabbages in considerable districts." He considered that the disease was "due to a soil Fusarium" but made no inoculation experiments. Aside from this observation his only contribution to our knowledge of the disease was in relation to its persistence in the soil; the organism resisted drying in the laboratory for three and one-half years. Woods ('99) showed that the characteristic symptom, the yellowing, was due to the presence of an increased amount of an oxidizing enzyme, peroxidase, in the diseased leaf tissue. Norton and Symons ('07) reported the presence of the disease in Maryland, but performed no experimental work.

Harter ('09), of the Bureau of Plant Industry, made inoculations of sterile soil with pure cultures of a *Fusarium* isolated from the stems of diseased cabbage plants. He was able to produce the characteristic symptoms in plants grown in that soil. In one trial, 83 per cent of these inoculations were successful; in a second, he reported that a large percentage of the plants showed typical symptoms, but no exact figure was given. He also made the statement that the fungus was a vascular parasite and formed microconidia in the vessels of the living plant. In addition to this paper Harter ('12) has published merely a popular account of the disease. Manns ('11) reported the disease as prevalent and destructive in Ohio but limited his work to field observations of a general nature.

Jones ('13, '14, '14[a]) in a series of papers reported the development of strains of winter and "kraut" types of cabbage which are highly resistant to the attack of this disease. These strains were developed by means of selection of sound

plants from badly diseased fields. In his last paper he reported that in the resistant strain 100 per cent formed commercial heads, or a yield of 18.8 tons to the acre; on the other hand, in the commercial strain used as controls, only 46 per cent of the plants lived and 24.2 per cent headed, or a yield of 2 tons to the acre.

These results show that, as far as practice is concerned, the disease has been controlled, but much remains to be done on the other aspects of the problem of the relation of host and parasite. Before discussing these phases, however, a brief description of the disease will not be out of place.

Symptoms of the Disease

The first evidence of the disease in the greenhouse is found on very young seedlings, often just after the appearance of the first true leaf, and is characterized by a rapid wilting of the cotyledons and dying of the roots while the stem is still turgid and, to all external appearances, normal. If, however, the conditions are not favorable for the attack of the fungus so early in the life of the host, the characteristic symptom —the yellowing of the leaf, to which the disease owes the name of "yellows"—is found. This yellowing may invade the entire plant, in which case wilting and death rapidly follow, or it may be confined to merely one side of the plant or leaf. If this one-sided invasion occurs, the plant or leaf ceases growth on the diseased side, but the green portions continue their development, bringing about a curvature of the plant or leaf toward the diseased area. This type is most frequently found on plants grown in the seed-beds in infected soil. These one-sided plants are usually stunted with the leaves loosely attached to the stem, so that they fall at the touch. If transplanted into the field such plants may die immediately, or if conditions are favorable for their development, they may live all summer, becoming stunted individuals with the lower leaves dying and dropping off, leaving a tuft of living leaves at the tip of a bare stalk. When healthy seedlings are transplanted to diseased soil the same characteristics occur; some plants die immediately—first, however, losing their chlo-

rophyll—while others become one-sided and stunted, but live throughout the summer. The latter rarely form heads.

CAUSAL ORGANISM

TAXONOMY

Cabbage yellows is caused by a soil fungus belonging to the genus *Fusarium*. The organism was first described by Wollenweber ('13) who, basing his classification on the work of Appel and Wollenweber ('10), placed it in the section *Elegans* and named it *Fusarium conglutinans* Wollenw. The description given by this author is as follows:

> '*Fusarium conglutinans* n. sp. differs from *F. orthoceras* * * * * in the absence of a wine-red color on rice which is a striking character of typical species of the section Elegans. * * * * Vascular parasite, cause of wilt disease of *Brassica oleracea* var. *capitata* (proved by Erwin F. Smith, L. R. Jones, L. L. Harter) in the United States of America.'

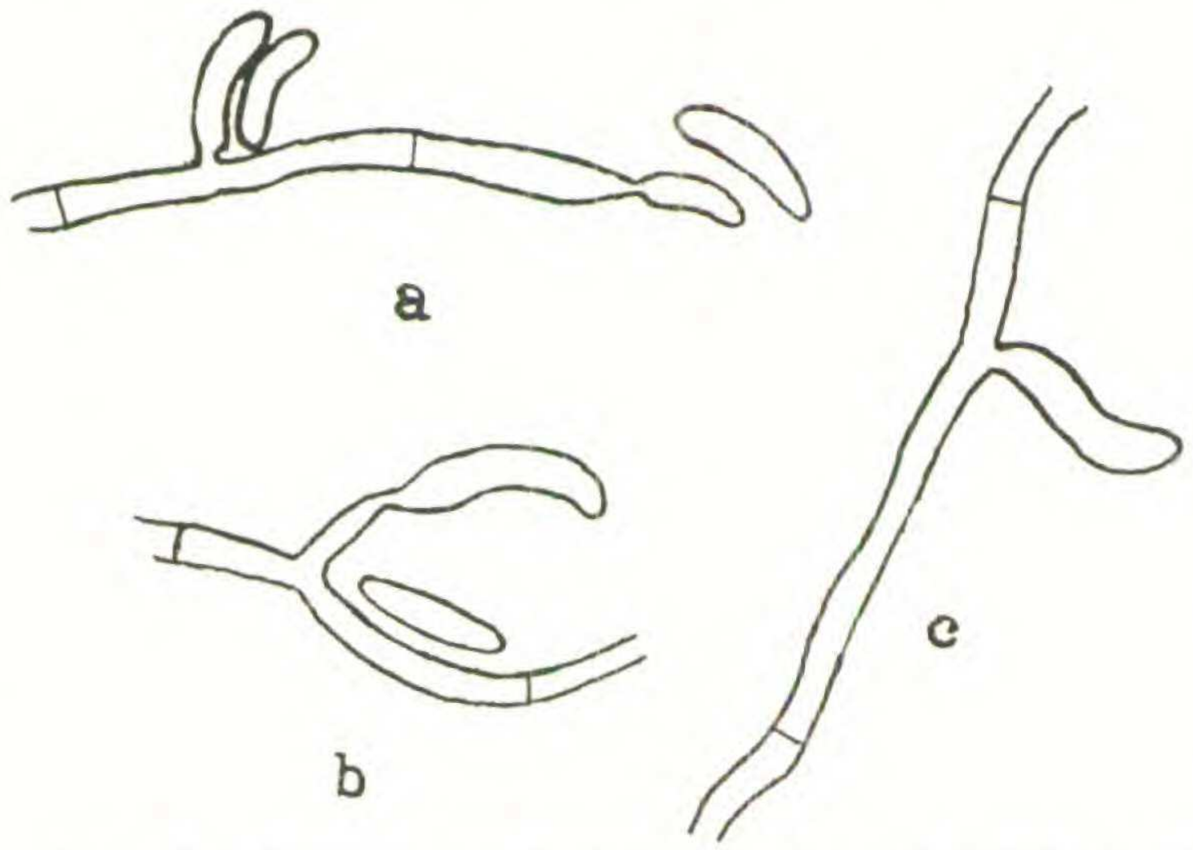


Fig. 1. Conidia production of *F. conglutinans* in Uschinsky's fluid after 48 hours: *a*, Culture V; *b*, Culture II; *c*, Culture I. Camera lucida sketch × 1000.

This description was based on a culture from the Laboratory of Plant Pathology of the University of Wisconsin. The same year Stevens ('13) ascribed the yellows to *Fusarium Brassicae* Thüm., citing Harter ('09) as his authority in spite of the following facts: first, that Harter specifically stated that he was working with an undescribed species and, second, that Wollenweber had included Harter's organism in his new species, *F. conglutinans*. Moreover, the organism that is parasitic on cabbage in the United States differs from *Fusarium Brassicae* Thüm. as described by De Thümen ('80)

in the following respects: *Fusarium Brassicae* forms *sporodochia,* while in *F. conglutinans* these are much reduced and usually not formed at all. *F. Brassicae* has conidia which are two-septate, while *F. conglutinans* has conidia, the majority of which are non-septate with a few one- and three-septate forms, two-septate spores not appearing. The main point of resemblance, from the description of *F. Brassicae,* is that the spore measurements fall within the same limits—a fact which, in view of the above differences, would scarcely suffice to put the two as synonymous.

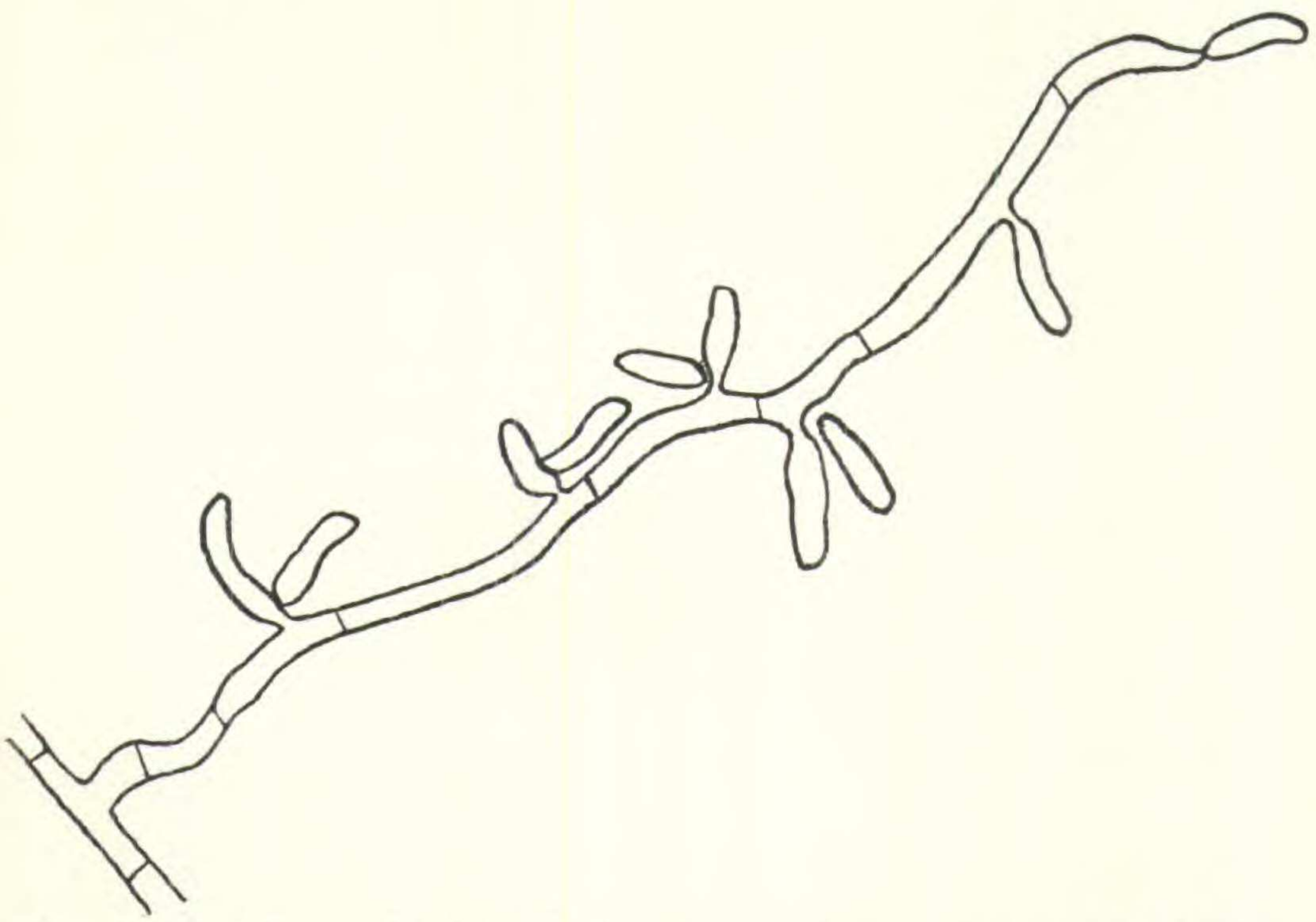
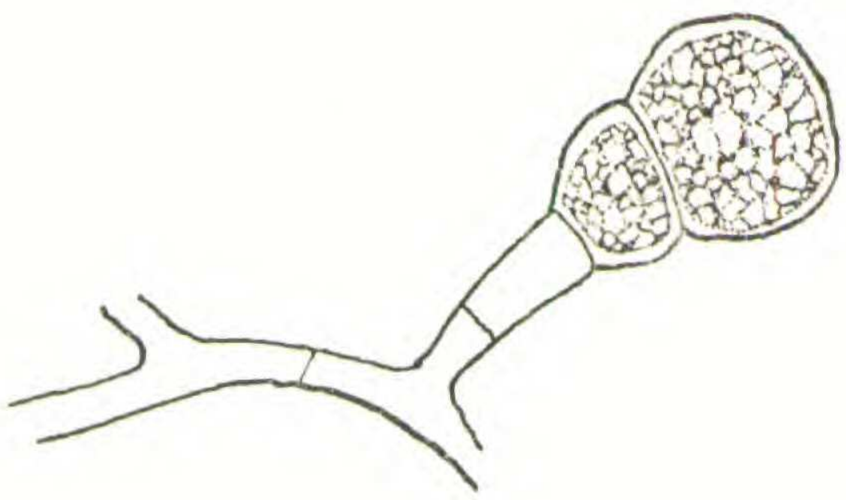


Fig. 2. Conidia production of *F. conglutinans* in Uschinsky's fluid after 48 hours. Culture I. Camera lucida sketch × 1000.

While the above description by Wollenweber is perhaps sufficient to differentiate *F. conglutinans* as a distinct form, it is hardly adequate as a diagnosis of the species; moreover, the question may be raised as to whether a physiological character, such as color production on a special medium, which has not been regarded as of specific rank in related genera, is sufficient basis for the establishment of a new species in the genus *Fusarium.* This, of course, introduces a new factor into the taxonomy of this genus, but the writer would hold it



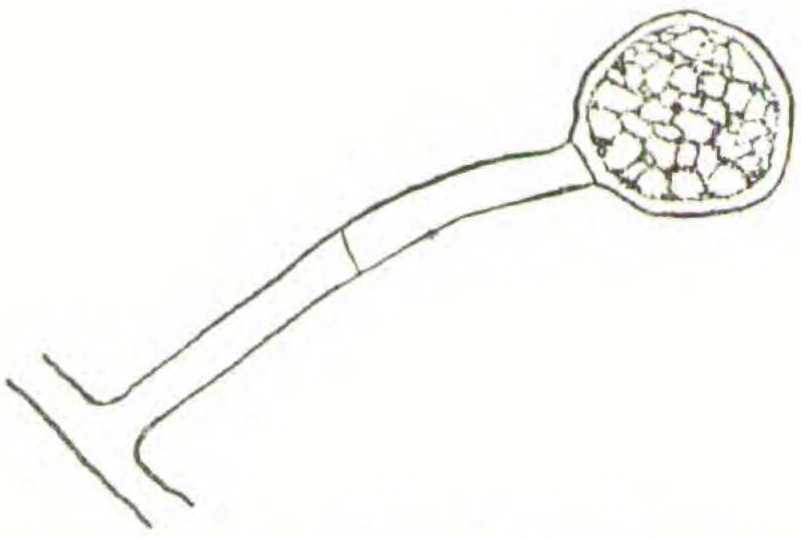


Fig. 3. Production of chlamydospores of *F. conglutinans* in Uschinsky's fluid after five days. Culture XI. Camera lucida sketch × 1000.

to be justifiable in such a group as the genus *Fusarium*, where a classification based on morphology alone has led and would continue to lead to confusion in many cases. That this character is constant in the case of *F. conglutinans*, there can be no doubt, but as additional evidence, some forty-three cultures of this organism have been maintained in the laboratory in connection with this work for a period of from six months to two years, and in no case did they produce red color on rice media, while cultures of *F. orthoceras*, carried as controls, did.

Moreover, on other media the organism maintained constant similarities with cultures from similar sources which on inoculation into the host produced the disease. The organisms were grown on potato hard agar, dextrose bouillon agar, soil extract agar, cooked potato plugs, cooked potato stems, and cooked rice. The mycelium in all cases grew well, giving a white fluffy growth at first, which gradually turned cream color, and in old cultures showed ochreous to brown strands in the aërial mycelium in the upper part of the tube. Spores of the "micro" type were found in all cultures in great abundance, especially during the early part of the growth of the cultures. The production of aërial mycelium was most abundant in those cases where the amount of carbohydrate in the substratum was greatest or most available.

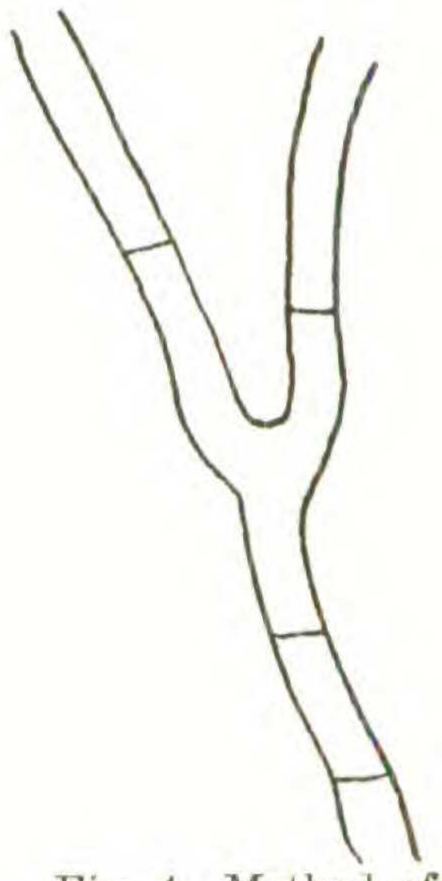


Fig. 4. Method of branching of mycelium of *F. conglutinans* in Uschinsky's fluid. Culture I. Camera lucida sketch × 1000.

After growth of a few weeks chlamydospores were found in most of the cultures, the microspores were beginning to become abnormal, and the few macrospores were also breaking down. The macrospores were found to be produced best on potato stems, next best on the potato agar, while very few appeared on cooked potato plugs and cooked rice.

Besides the tube cultures, hanging-drop cultures in a modified Uschinsky's fluid[1] were observed. In this medium the fungus produced microspores abundantly in cultures only two days old when kept at room temperatures. Chlamydospores were found to begin to form in cultures but five days old, although they did not mature in so short a time. Usually the first chlamydospores occurred terminally; later other parts of the mycelium rounded up to form the intercalary spores.

A revised description of the fungus is as follows:

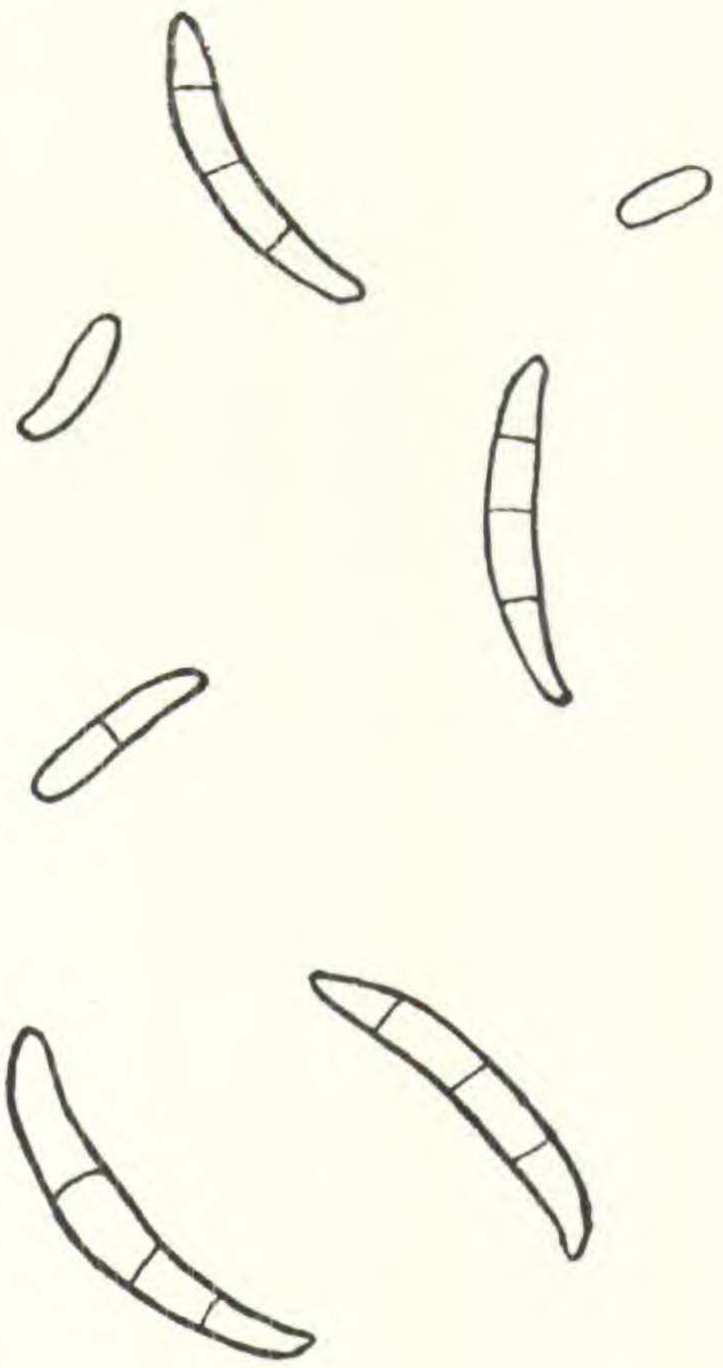


Fig. 5. Conidia of *F. conglutinans*. Culture LVI on potato stem. Camera lucida sketch × 800.

Fusarium conglutinans Wollenw.

Sporodochia lacking or greatly reduced; pionnotes never present. Conidia borne on short conidiophores strewn throughout the mycelium, majority non-septate, a few one-septate and three-septate. The non-septate conidia ovoid to ellipsoidal, hyaline, 2.5–4 × 6–15μ, the majority being 2.5–3 × 7–10μ. One-septate conidia hyaline, cylindrical, with

[1] This medium was a modification of the standard Uschinsky's fluid, and was made up as follows:

Water, distilled	1000 grams	Magnesium sulphate	0.3 grams
Glycerin	30 grams	Dipotassium phosphate ..	2 grams
Sodium chloride	5 grams	Ammonium tartrate	6 grams
Calcium chloride	0.1 grams	Sodium asparaginate	3 grams

The solution was sterilized at ten pounds pressure for twenty minutes in the autoclave.

rounded ends, long axis slightly bent, dimensions $4 \times 19\mu$. Three-septate conidia fusiform, hyaline, with both end-cells tapering and with rounded tips, no sharply differentiated foot, dimensions $3.5–5.5 \times 25–33\mu$. Conidia with higher septation very rare.

In culture aërial mycelium white at first, becoming cream-colored and finally showing a development of ochreous strands of thallo-plectenchymatic tissue throughout, but no sclerotia. Grows well on potato agar, dextrose bouillon agar, Uschinsky's fluid, cooked rice, cooked potato plugs, and potato stems. On no medium is there any color production except the slight yellowing spoken of above. In older cultures terminal or intercalary chlamydospores are produced. They are usually one-, sometimes two-celled, spherical to ovoid with a thick irregular wall, frequently slightly colored. Dimensions $7–12 \times 7–15\mu$.

The fungus is found in the soil and is a vascular parasite attacking cabbage, *Brassica oleracea* var. *capitata,* causing the yellows, or wilt disease. It has also been isolated as a saprophyte from China aster and tubers of potato (Lewis, '13).[1]

TEMPERATURE STUDIES

In view of the later work in regard to the relation of temperature to the occurrence of the disease, it will be well to discuss briefly this relation for the fungus in pure culture, both as to growth and germination of conidia. The latter will be considered first.

In order to obtain spores of the fungus free from pieces of mycelium, a bit of mycelium was placed in a hanging-drop of Uschinsky's fluid in a Van Tieghem cell which was partially filled with Uschinsky's fluid below. The spores were formed abundantly at room temperatures in forty-eight hours and allowed to drop into the lower liquid from which they were

[1] Through the kindness of Dr. W. J. Morse, of the Maine Agricultural Experiment Station, transfers of these two strains of *Fusarium conglutinans* were obtained, and inoculations made on February 2, 1915, on five plants each, and again on February 26, 1915, with the strain from aster on ten plants; both gave negative results. This would indicate that they belonged to a saprophytic strain, although the results might be due to the fact that the fungi had been so long in culture that they had lost their virulence, or to the small number of trials made. Control cultures of this fungus from cabbage, however, gave 80 per cent infection in the first case and 100 per cent in the second trial.

transferred to other Van Tieghem cells by means of a sterile pipette. They were immediately placed in the incubators at the desired temperatures, and observed at intervals for germination. All observations were made in duplicate, and the trials were repeated twice to verify them. The temperatures used were 8–10°C., 10–12°C., 16°C., 21°C., and 33°C. The results are brought together in table I.

TABLE I

GERMINATION OF CONIDIA OF FUSARIUM CONGLUTINANS AT VARIOUS TEMPERATURES

Hours of exposure	Germination (+) at the following temperatures									
	8–10° C.		10–12° C.		16° C.		21° C.		33° C.	
	Trial no.		Trial no.		Trial no.		Trial no.		Trial no.	
	1	2	1	2	1	2	1	2	1	2
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	+	+
6	—	—	—	—	—	—	—	—	+	+
8	—	—	—	—	+*	+*	+	+	+	+
12	—	—	—	—	+	+	+	+	+	+
24	—	—	—	—	+	+	+	+	+	+
36	—	—	—†	—†	+	+	+	+	+	+
72	—	—	—	—	+	+	+	+	+	+

* In a later test in which more frequent observations were made, conidia at this temperature (16°C.) germinated 12 hours after the beginning of the exposure.

† At this temperature (10–12° C.) spores were found to germinate after 36 hours in a later experiment. The number that germinated, however, was very small and the growth exceedingly slow.

It will be noted that, as was to be expected, spores of the fungus germinated best at the higher temperatures of the experiment, although they were able to grow slowly at the lower temperatures. These facts are further borne out by the growth of the fungus on potato agar. Transfers of a bit of the mycelium from a rapidly growing culture were placed in the center of plates of potato hard agar, and the plates were then placed in the incubators at the desired temperatures.

Three plates were carried at each temperature, and measurements of the growth of the colonies of the mycelium were made each day for ten days, after which time the experiment was discontinued because of the contamination of some of the plates and the drying out of others. The results are given in table II.

TABLE II

GROWTH OF FUSARIUM CONGLUTINANS AT VARIOUS TEMPERATURES

Age of colony in days	Diameter of colony in cm. at various temperatures											
	4–8° C.			18° C.			21–22° C.			25° C.		
	Plate no.			Plate no.			Plate no.			Plate no.		
	1	2	3	1	2	3	1	2	3	1	2	3
1	....	....	....	....	....	....	0.9	0.9	0.6	0.7	1.1	0.1
2	....	....	....	0.1	0.1	0.1	1.6	1.5	1.2	1.8	2.0	1.1
3	0.1	0.1	0.1	0.6	0.6	0.6	2.0	2.0	1.8	2.0	2.2	1.7
4	0.2	0.2	0.1	1.0	0.9	0.9	3.0	2.8	2.4	2.9	3.2	2.9
5	0.4	0.5	0.4	1.2	1.1	1.2	3.6	3.4	3.0	3.4	−*	3.1
6	0.5	0.7	0.5	1.4	1.2	1.3	4.0	3.8	3.4	3.8	....	3.7
7	0.7	0.8	0.9	1.5	1.3	1.5	4.5	4.3	−*	4.6	....	4.4
8	1.1	1.1	1.1	1.9	1.7	1.7	5.1	4.9	....	5.1	....	5.3
9	1.4	1.5	1.3	2.0	1.8	1.8	5.5	5.4	....	5.6	....	6.0
10	1.6	1.7	1.4	2.2	2.2	1.9	6.2	6.0	....	−*	....	6.6
Average growth per day	0.16	0.17	0.14	0.22	0.22	0.19	0.62	0.60	0.56	0.62	0.80	0.66
Average for each series	0.16			0.21			0.59			0.69		

*Contaminated.

If the growth of *Fusarium conglutinans* be compared with that of some of our more common saprophytic forms as, for example, *Penicillium glaucum*, or *Aspergillus niger*, as reported in the literature, it will be noted that, while the optimum of these forms is also high, they can grow better than *F. conglutinans* at the lower temperatures. In other respects the curves of growth of these forms would approximate one another very closely.

No attempt was made to find the maximum and minimum growth temperature for this fungus, because the object of the work was to find, if possible, an explanation for the fact that yellows occurred in the host at high temperatures rather than at low. This relation will be discussed later when a full review of the points involved will be taken up.

Inoculation Experiments

The first inoculation experiments were tried during the summer of 1912. On July 17 five flats of soil were planted to cabbage; three contained soil brought from the experimental plot at Racine and two, normal greenhouse soil. One of the latter was left as a control, and the other was inoculated with spores from a pure culture of the fungus. These flats were kept shaded on the north side of some shrubbery in the pathological garden and no typical yellows had appeared by September 7, when they were discarded. On August 23 five plants in the pathological garden were inoculated by placing mycelium of a rapidly growing culture in contact with the roots. No disease was found up to October 22, when frost killed the plants. The seedlings were two weeks old at the time of inoculation. Again, on September 10, thirty healthy plants were transferred to three flats of soil brought from the experimental plots at Racine, but no disease was found in any of the flats by December 2.

On January 6, 1913, twelve pots of sterile soil were planted to cabbage and inoculated by stirring cultures of *Fusarium* into the pots. Twelve pots of diseased soil from the experimental plot, eight of sterilized soil, and four of normal undiseased soil were planted as controls. Spores were abundant in all the cultures used. No yellows had appeared by April 29, and the plants were then pulled and the pots replanted. Instead of keeping this second lot in the open greenhouse,

however, they were placed under a glass, such as is used in a forcing-bed, thereby giving a higher temperature than could be attained in the open house. On January 14 the plants in one pot of inoculated soil showed the characteristic symptoms, and on July 10 the plants in a second pot had succumbed. On July 12 a third pot contained plants showing the disease. Damping off due to *Rhizoctonia* interfered with the value of this trial. The diseased plants were plated out on potato hard agar in all cases, and the typical *Fusarium* found to be present. As will be noted, it was only after the temperature of the pots had been raised that there was any occurrence of the disease. Curves showing the temperature attained by placing the plants under the glass are given in fig. 6.

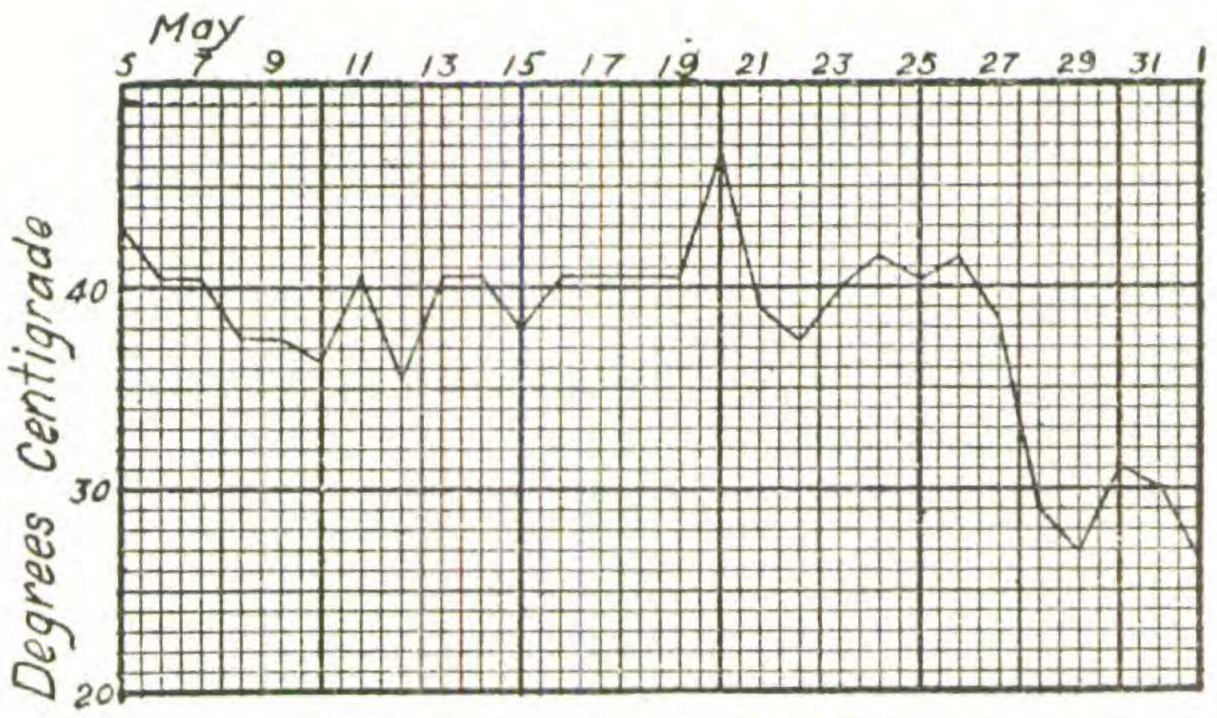


Fig. 6. Diagram showing temperature under glass in greenhouse during inoculation experiments, May 5–31, 1914.

On May 10, 1913, another inoculation experiment was started with seedlings grown in normal greenhouse soil. The plants used were about ten days old — just showing the first true leaf. Five pots of soil were used, five seedlings in each pot. Pot 1 was left as a control, while the roots of the plants in the other pots were dipped into a suspension of spores from a pure culture and immediately planted. On May 13 they had all recovered from the effects of transplanting and were in good

condition. On July 10 symptoms of yellows showed in pots 4 and 5; on July 14 the plants in pot 3 became diseased, but no disease was found in pots 1 or 2. The plants were plated on potato hard agar, and *F. conglutinans* was recovered from plants from pots 3, 4, and 5. None was found on seedlings plated from pots 1 and 2. Culture V, which upon reinoculation again produced the disease, was one of these cultures (table v).

On May 27, 1913, a more extensive inoculation experiment was started; part of the pots containing the plants were placed in the greenhouse and part in the pathological garden. As the greenhouse was not heated, the difference of conditions was not noticeable, and, therefore, the results are combined. For this trial twenty pots were planted to cabbage. These pots were in duplicate, ten being placed in the greenhouse and ten in the soil of the garden. The treatment of the soil in these pots was as follows: Eight pots contained soil from the experimental plots at Racine, and eight others normal greenhouse soil. These sixteen containers were sterilized in an autoclave at eleven pounds pressure for four hours. Four of each of them were used as controls, four were inoculated with pure cultures of *F. conglutinans,* and the remaining four were inoculated with wilted leaves of the diseased plants. Two pots of normal greenhouse soil and two of normal diseased soil were added to the series as controls. The cultures were added by mixing them with the surface soil in the pots. The seed used were not good, and, therefore, the plants did not come up well, so that in the following pots there were no plants: one pot of diseased soil sterilized but not inoculated; two pots of diseased soil sterilized and inoculated with pure cultures; two of diseased soil sterilized and inoculated with the leaves; one pot of normal greenhouse soil not inoculated; and one of sterilized greenhouse soil inoculated with leaves. The results of this experiment are given in table III.

TABLE III

RESULTS OF INOCULATIONS WITH PURE CULTURES OF FUSARIUM CONGLUTINANS

Pot no.	Kind of soil	Treatment of soil	Plants per pot		
			Total no.	No. diseased	Percentage diseased
1	Infected	Sterilized	7	0	0
2	Infected	Sterilized	0	.....	.....
3	Infected	Ster'z'd and inoc'l'd	4	3	75
4	Infected	Ster'z'd and inoc'l'd	1	1	100
5	Infected	Normal	5	4	80
6	Infected	Normal	6	6	100
7	Uninfected	Normal	1	0	0
8	Uninfected	Normal	0	.....	.....
9	Uninfected	Ster'z'd and inoc'l'd.	3	1	33
10	Uninfected	Ster'z'd and inoc'l'd	5	5	100
11	Uninfected	Ster'z'd and inoc'l'd	1	1	100
12	Uninfected	Ster'z'd and inoc'l'd	2	1	50
13	Uninfected	Sterilized	6	0	0
14	Uninfected	Sterilized	4	0	0
15	Uninfected	Ster'z'd and infect. leaves	2	1	50
16	Uninfected	Ster'z'd and infect. leaves	0	.....	.....

The results are not as conclusive as they might be, however, as the number of plants used was very small due to the poor seed mentioned above. The pots in which no plants grew in either of the duplicates were omitted. An idea of the appearance of the plants at the time of making counts may be had from pl. 1, fig. 2. It should be noted that these successful inoculations were made at the warmest time in the summer. Plates were made on July 14 from plants in each pot, and in all cases the diseased seedlings gave pure cultures of *F. conglutinans,* while those from the controls remained sterile. Culture X was one of these and upon reinoculation again produced the disease (table v).

On June 12 the above experiment was repeated, using three flats of soil (from the experimental field), two of which had been sterilized at eleven pounds pressure for four hours on two successive days, the other remaining untreated. One of

the sterile flats was inoculated by stirring into its surface ten pure cultures of *F. conglutinans* which were sporulating abundantly. Then all the flats were planted. As an additional control, a flat of normal greenhouse soil was placed in the series. On July 7 yellows began to appear in the sterilized inoculated flat and continued to spread until July 16, when the experiment was concluded by making plates from the plants from the sterilized inoculated, and the sterilized flats. *F. conglutinans* was found as the cause of the yellowing in all the plants, while the control plants remained sterile. This is shown well in pl. 2, figs. 15 and 16. Exact counts were not made.

Again, on June 29, four pots of sterile greenhouse soil were inoculated with cultures of *F. conglutinans,* and on July 24 yellows was found in all four of the pots. On July 11 inoculations of individual plants were repeated by dipping wounded plant roots into suspensions of the spores of the fungus in sterile water. Adequate controls were included in this series and in all cases the control plants remained healthy, while among the inoculated plants, 50 per cent of the individuals showed the characteristic symptoms on July 24, when the experiment was discontinued.

VIRULENCE OF CULTURES

Pure cultures of *F. conglutinans* vary greatly in their virulence, and the cause of this variation is not certain. From inoculation experiments it would seem that, in general, the longer the organism has been carried in culture the greater is the probability that it has lost its virulence. On the other hand, drying in culture seems to have little or no ill effect on the virulence of the organism.

The susceptibility of the host must also be considered as an important factor when the fungus-host relation hangs in such a delicate balance, and the source of the culture is always

of importance also. The medium upon which the culture is grown and the state of the mycelium and spores have been pointed out by Wollenweber as important factors in other species of *Fusarium* which produce plant disease, and doubtless they bear their part in the irregularity of the results presented here.

In a series of inoculation experiments made at the Missouri Botanical Garden, recently isolated cultures were used as sources of infection. The cultures were grown on cooked potato stems, and inoculation was effected by placing a bit of the culture tissue in contact with a wound on the hypocotyl of the plant. The plants were in the cotyledonous stage, and after inoculation were placed in normal uninfected greenhouse soil. Five seedlings were placed in each pot. Table IV gives the results of the experiments.

TABLE IV

PRELIMINARY STUDY OF VARIATION OF VIRULENCE OF FUSARIUM CONGLUTINANS IN PURE CULTURE

Culture number	Date of isolation	Total no. of plants	No. of diseased plants	Per cent of diseased plants
XVI	7/14/13	40	30	75.0
LV	11/17/14	15	13	86.6
LVI	2/1/15	5	5	100.0
Control	.....	10	0	0.0

It will be noted that where the larger number of plants was used the percentage of infection fell. This result might have been expected if the age of the cultures used and the great variation in susceptibility of the host plant were taken into consideration, but to gather more data on these points a trial was made with a large series of cultures that had been isolated at various times and also from various sources.

Table v gives the data on the inoculation experiment which was carried out similarly to the one just reported.

TABLE V
RESULT OF INOCULATION EXPERIMENT SHOWING VARIATIONS IN VIRULENCE OF FUSARIUM CONGLUTINANS IN PURE CULTURE

Culture number	Species	Source	Date of isolation	Pathogenicity					
				Damped off		Yellowed		Healthy	
				No.	Per cent	No.	Per cent	No.	Per cent
I	*F. conglutinans*	Cabbage	5/16/13	0	0	0	0	5	100
II	*F. conglutinans*	Cabbage	6/12/13	0	0	0	0	5	100
V	*F. conglutinans*	Cabbage	5/10/13	1	20	3	60	1	20
VI	*F. conglutinans*	Cabbage	5/10/13	1	20	0	0	4	80
IX	*F. conglutinans*	Cabbage	7/14/13	0	0	0	0	5	100
X	*F. conglutinans*	Cabbage	7/14/13	1	20	1	20	3	60
XI	*F. conglutinans*	Cabbage	7/14/13	5	100	0	0	0	0
XIII	*F. conglutinans*	Cabbage	7/14/13	0	0	2	40	3	60
XIV	*F. conglutinans*	Cabbage	7/14/13	0	0	0	0	5	100
XVI	*F. conglutinans*	Cabbage	7/14/13	0	0	4	80	1	20
XVII	*F. conglutinans*	Cabbage	7/28/13	0	0	0	0	5	100
XVIII	Undetermined	Cauliflower	7/26/13	0	0	0	0	5	100
XIX	*F. conglutinans*	Cabbage	5/ 6/13	0	0	3	60	2	40
XX	*F. conglutinans*	Cauliflower	7/26/13	0	0	2	40	3	60
XXI	*F. conglutinans*	Cabbage	7/26/13	0	0	0	0	5	100
XXII	*F. conglutinans*	Cabbage	7/26/13	0	0	0	0	5	100
XXIII	*F. conglutinans*	Cabbage	7/26/13	0	0	0	0	5	100
XXIV	*F. conglutinans*	Cabbage	7/26/13	0	0	0	0	5	100
XXV	*F. conglutinans*	Cabbage	7/27/13	4	80	1	20	0	0
XXVI	*F. orthoceras*	Cabbage	5/ 6/13	0	0	0	0	5	100
XXVII	Undetermined	Cabbage	8/ 4/12	0	0	0	0	5	100
XXVIII	*F. conglutinans*	Cabbage	8/ 4/12	0	0	0	0	5	100
XXIX	Undetermined	Cauliflower	8/24/12	0	0	0	0	5	100
XXX	*F. conglutinans*	Cabbage	6/28/12	0	0	3	60	2	40
XXXII	*F. conglutinans*	Cabbage	6/28/12	1	20	4	80	0	0
XXXIII	Undetermined	Cabbage	6/28/12	2	40	0	0	3	60
XXXV	*F. conglutinans*	Cabbage	8/24/12	1	20	0	0	4	80
XXXVI	*F. conglutinans*	Cabbage	6/24/12	2	40	0	0	3	60
XXXVII	*F. conglutinans*	Cabbage	6/28/12	1	20	1	20	3	60
XXXVIII	*F. conglutinans*	Cabbage	6/28/13	0	0	0	0	5	100
XXXIX	*F. conglutinans*	Cabbage	6/28/13	1	20	0	0	4	80
XL	*F. conglutinans*	Cauliflower	1/ 3/14	5	100	0	0	0	0
XLII	*F. conglutinans*	Cabbage	2/14/14	2	40	2	40	1	20
XLIII	Undetermined	Aster	.....	1	20	0	0	4	80
XLIV	*F. conglutinans*	Aster*	.....	0	0	0	0	5	100
XLV	*F. conglutinans*	Potato*	.....	3	60	0	0	2	40
XLVI	*F. conglutinans*	Cabbage	4/13/14	1	20	0	0	4	80
XLVII	*F. conglutinans*	Cabbage	3/12/14	0	0	3	60	2	40
LI	*F. conglutinans*	Cabbage	5/ 6/14	0	0	0	0	5	100
LII	*F. conglutinans*	Cabbage	5/ 6/14	0	0	2	40	3	60
LIII	*F. conglutinans*	Cabbage	5/18/14	0	0	2	40	3	60
LIV	Undetermined	Cabbage	9/24/14	0	0	0	0	5	100
LV	*F. conglutinans*	Cabbage	11/17/14	0	0	4	80	1	20
LVI	*F. conglutinans*	Cabbage	2/ 1/15	0	0	5	100	0	0
Control	No fungus	.....	.....	1	10	0	0	9	90

*Isolated by Lewis ('13) at the Maine Agricultural Experiment Station and determined by H. W. Wollenweber, Bureau of Plant Industry, Washington, D. C.

The cultures were all prepared in the same way for the experiment. They were all grown on cooked potato stems, and were of the same age. Each culture was used to inoculate five plants by inserting a bit of the mycelium into the hypocotyl of young seedlings still in the cotyledonous stage.

The previous history of the cultures, of course, differed for the individual. Cultures VI, XVII, and XIX had been allowed to dry out on potato hard agar for fourteen months, that is, from July 14, 1913, to September 26, 1914, and then were transferred to cooked potato stems. On January 12, 1915, they were again transferred to fresh cooked potato stems, and these cultures were used in the experiment. Although two of them (VI and XVII) apparently lost their virulence, the third (XIX) retained its ability to attack the host even after this severe drying. Other strains which had not been allowed to dry out but which were kept on fresh media, possessed no greater virulence, nor did any greater percentage of them exhibit pathogenicity.

The length of time the organism has been in culture seems to be a more important factor; for cultures isolated late in 1914 showed proportionally a larger number virulent than did those isolated at earlier dates. In addition, the more recent isolations showed the greater virulence. That this is not invariable, however, is shown by the fact that many of the cultures first isolated still retained their virulence, viz., XXX, XXXII, XXXVII, all three of which were isolated on June 28, 1912.

The source of the culture seems to have greater influence. Of the six strains of *F. conglutinans* isolated from cauliflower grown in diseased soil and apparently attacked with yellows, but one (XX) showed any ability to infect cabbage and that only to a limited extent. Strains from aster and potato, kindly furnished by Dr. W. J. Morse of the Maine Agricultural Experiment Station, also gave negative results when inoculated into cabbage. *F. orthoceras,* which had been isolated from the stem of a diseased cabbage plant, was introduced into the series as a control. A number of undetermined Fusarium cultures which had been isolated from cabbage, cauli-

flower, and China aster were added for the same reason. None of these latter were capable of infecting the living cabbage plant.

SUSCEPTIBILITY OF HOST

That the susceptibility of the host plant must also play an important part in this question of inoculation is shown by the fact that so few of the cultures gave a perfect (100 per cent) infection, although the inoculations were made with parts of the same culture on plants from the same pot and under as identical conditions as possible.

Further evidence on this point was also shown when the difference in the length of the incubation period of any one culture was noted on plants of the same variety and age. For example, in the last experiment observations were made daily in the greenhouse, and the condition of the plants noted. The results of these observations are brought together for a few of the cultures in table VI.

TABLE VI

RESULTS OF OBSERVATIONS ON INDIVIDUAL SUSCEPTIBILITY AS SHOWN BY THE INCUBATION PERIOD UPON INOCULATION

Culture no.	Number of diseased plants in each pot at the various days of incubation						
	15	16	17	19	23	40	50
XVI	1	2	3	3	3	3	4
V	.....	2	2	2	3	3	3
XIX	.....	.....	2	3	3	3	3
XLII	.....	.....	1	1	4	4	4
XXX	.....	.....	.....	2	3	3	3
XXXII	.....	.....	.....	1	2	3	4
Control	.....	.....	.....	.....	.....	.....	.....

Thus it is shown that not only were some virulent cultures slower in taking effect than others, but that the individual plants were markedly different in their ability to resist the fungus. Although what constitutes such resistance has not been worked out, a little evidence gathered during these investigations may well be presented here.

In the field it was noted that the plants of the resistant strains of cabbage were, as a rule, larger than plants of the commercial strains of the same age. The first year it was thought that this difference in size might be due to crowding in the seed-bed of the plants of the commercial strain, chiefly because the amount of available seed of the resistant varieties was limited, while that of the commercial strain was plentiful. When this fact repeated itself over three years, experiments in the laboratory were run to account for the difference. Seeds were placed between moist filter paper in petri dishes and allowed to germinate. Twenty-five seeds were placed in each dish, and two dishes of each strain, VIII a-16 and commercial Danish Ball-head, were germinated. After wetting up the filter paper with distilled water the dishes were all placed in an incubator at 22°C. The seeds of the resistant strain germinated twelve hours before those of the commercial varieties, three days after the beginning of the experiment. Plate 2, figs. 5 and 6 give an idea of the appearance of the seedlings at this time, under similar conditions of moisture and temperature.

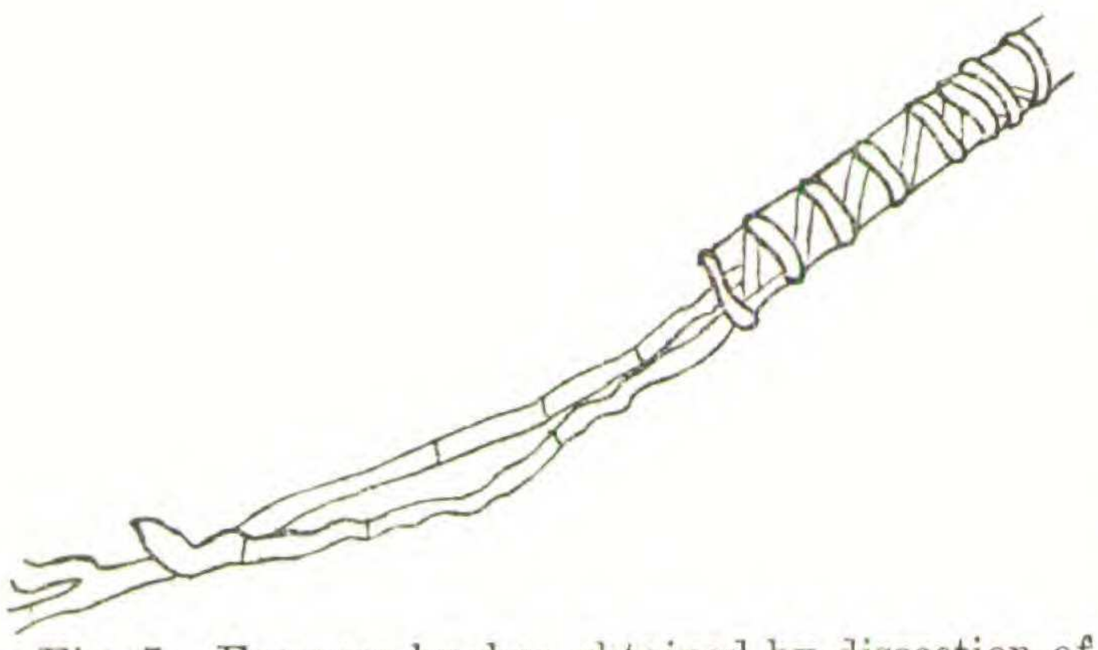


Fig. 7. Fungous hyphae obtained by dissection of diseased stem after boiling in KOH solution. Camera lucida sketch.

This characteristic of growth suggested that there might be a considerable difference in osmotic pressure between the root cells of the two strains, and experimental work was undertaken to determine whether the threshold of plasmolysis of the two strands differed toward NaCl solution as a plasmolytic agent. Two trials were made using the root-hairs as indicators, but in neither case was any difference between the threshold of plasmolysis of the resistant strain and that of the susceptible strain found.

Host Relations

MORPHOLOGY

The distribution of the fungus in the living host tissues is limited to the vascular bundles. This fact was first shown in making plates from old stems of diseased plants. The stems were cut cross-wise in thin sections and, after sterilization in hydrogen peroxide for five minutes and washing in sterile water, were laid on the surface of poured plates of potato hard agar. Invariably the first growth of the mycelium appeared from the fibro-vascular ring (pl. 2, fig. 14). Upon dissection of diseased seedlings the hyphae were demonstrable

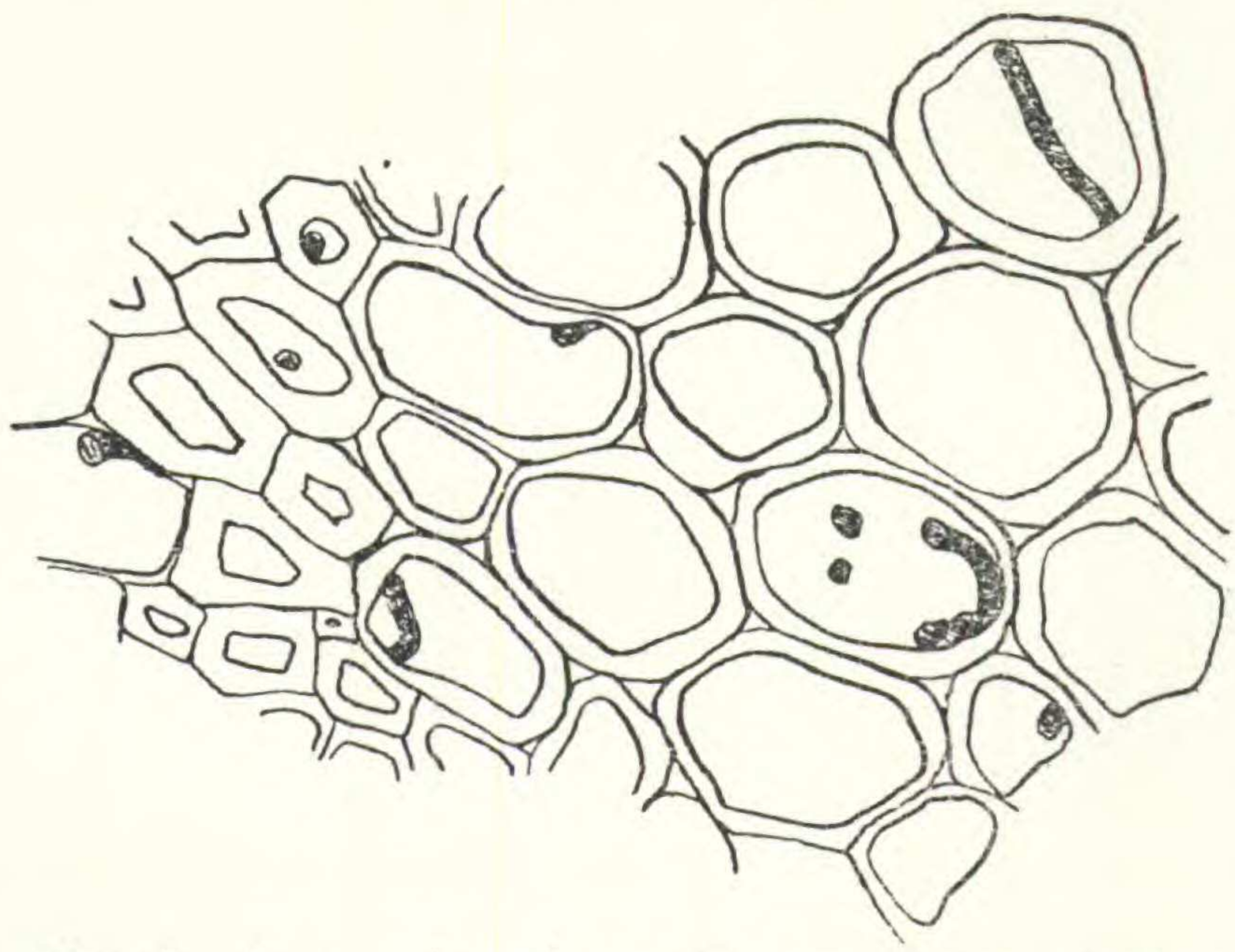


Fig. 8. Cross-section of vascular bundle from diseased cabbage stem, showing distribution of fungus in vessels. Note preponderance of cut ends of hyphae. Stained with Pianeze IIIb. Camera lucida sketch × 1000.

traversing the lumina of the bundles longitudinally. The stems were first boiled for five minutes in a 5 per cent potassium hydroxide solution and then dissected under a hand lens. The final examination was made under the compound microscope. In no case was a very large amount of mycelium found in any single vessel (fig. 7).

In later work the diseased stems were imbedded in paraffin in the usual manner, after fixing in Gilson's solution, and stained with Pianeze IIIb, as recommended by Vaughan ('14). The fungus stained a deep red, while the host tissue was col-

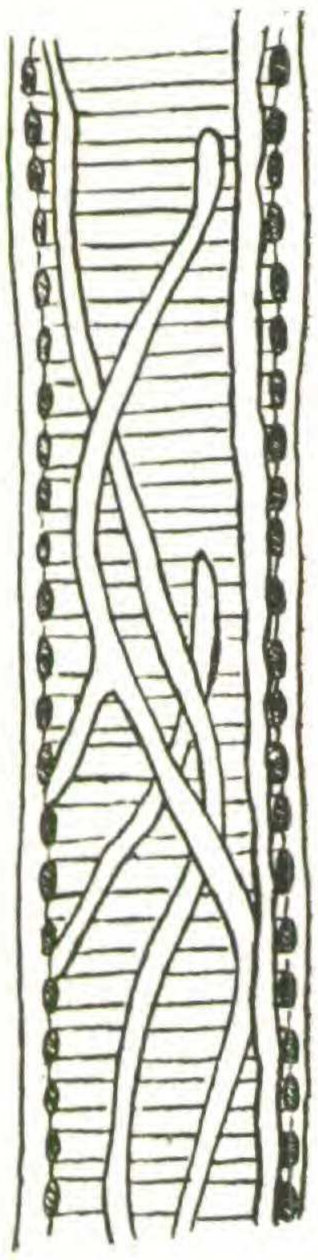


Fig. 9. Longitudinal section of vessel in diseased cabbage stem, showing vegetative hyphae. Stained with Pianeze IIIb. Camera lucida sketch × 1000.

ored green. Longitudinal sections showed the hyphae of the fungus running longitudinally in the lumina of the spiral vessels and the bast fibres. Cross-sections showed only the cut ends of the fungus. Drawings illustrating these facts are shown in figs. 8 and 9 which were made with the aid of a camera lucida. It was found that besides the purely vegetative hyphae, the fungus produced conidia in the vessels of the host (fig. 10). Those spores observed in the host tissue were all of the unicellular type.

All the evidence shows that the fungus attacks the root first, but just how remains to be worked out. After entering the host, it is confined to the vascular system. The fungus was never isolated from the stem until after a marked yellowing of the leaves appeared, although it was always present in the tissues before they had been killed. This fact is brought out in pl. 2, fig. 12 in which is shown a branched plant, one branch of which was attacked, while the other remained healthy in appearance. The leaf at *E* was still alive although one side of it was yellow. The stem of the plant appeared normal externally. The fungus, however, was isolated from the stem below the branching and at the points *D* and *E* on the diseased branch, while the parts at *B* and *C* on the other branch remained sterile. The plate made from this plant is shown in pl. 2, fig. 13.

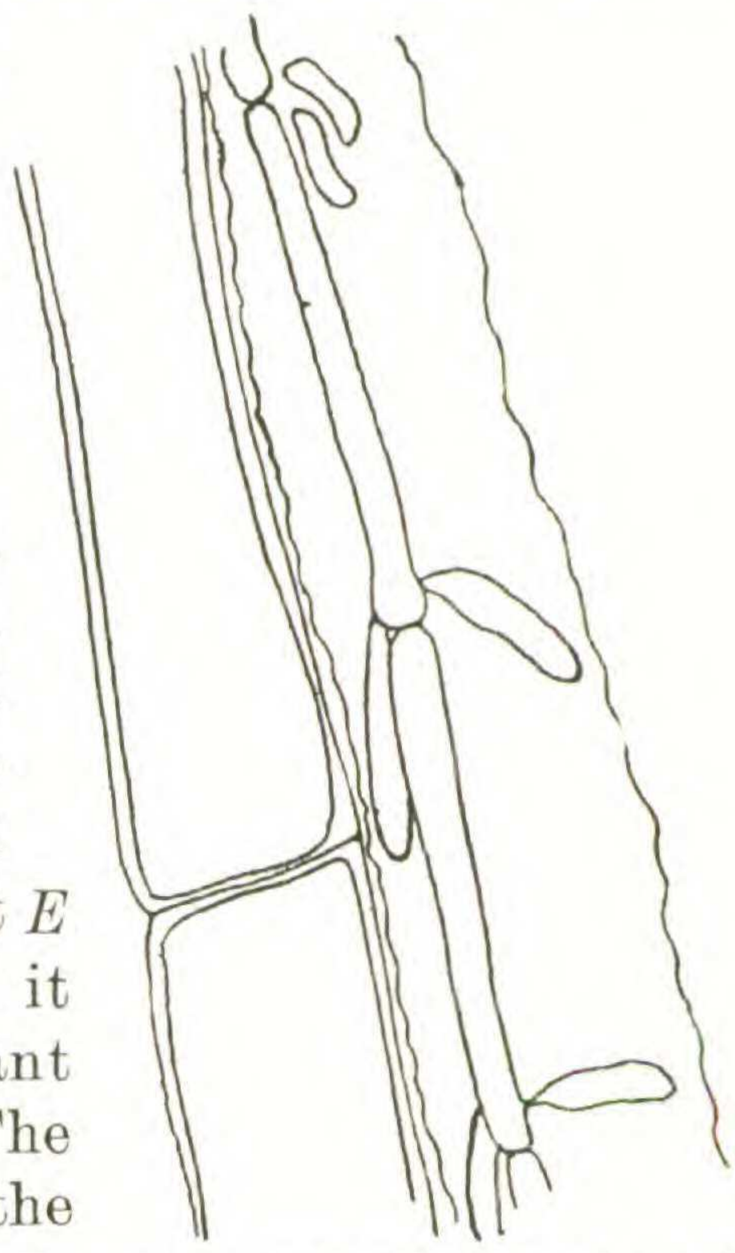


Fig. 10. Longitudinal section of vessel in diseased cabbage stem, showing production of conidia. Stained with Pianeze IIIb. Camera lucida sketch × 1000.

After the death of the host the fungus traverses all the tissues, sporulating at the surface and within the host also. In this way the fungus is able to return to the soil. Whether it may winter over in the host tissue was tested by marking plants which have been killed by the yellows in the field in 1913, and then bringing these plants into the laboratory in the spring of 1914. The stumps were first freed from the soil by brushing them under water and then washing in running water for fifteen minutes. After this washing the stalks were divided into equal portions and placed in two flats of sterile greenhouse soil (sterilized in the autoclave at eleven pounds pressure for five hours) and left for twenty-four days, after which time the flats were planted to cabbage on June 3, 1914. Yellows was found in both flats on July 6, 1914, showing that the fungus was able to get back into the soil from these stems, or that the roots coming in contact with the stems were attacked.

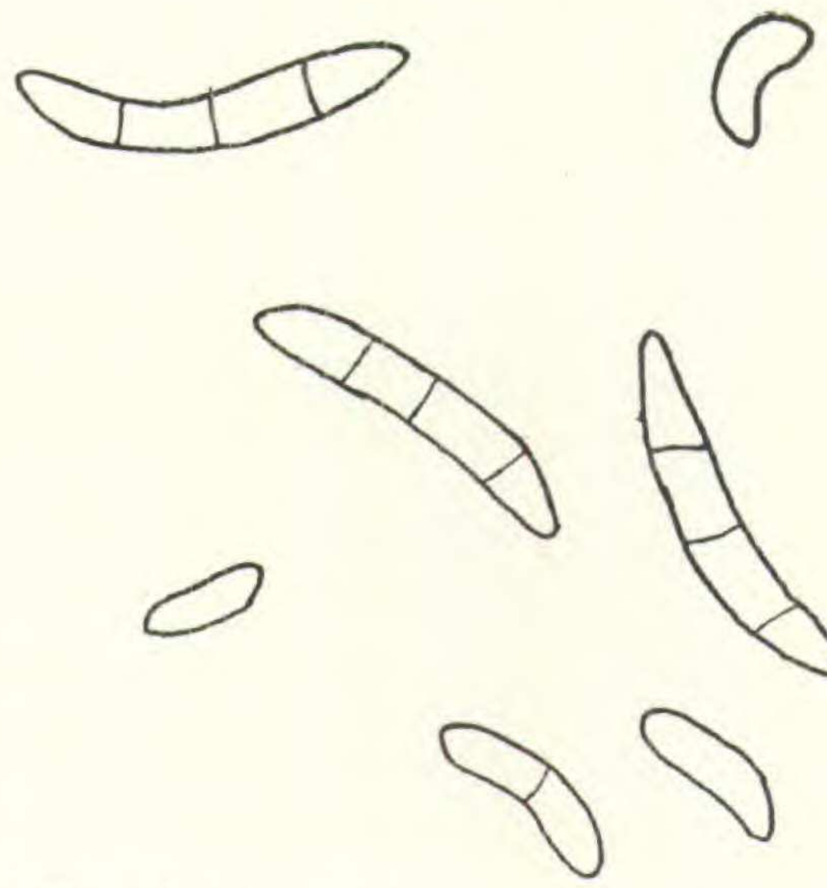


Fig. 11. Conidia obtained from over-wintered cabbage stem in spring of 1915. Camera lucida sketch × 800.

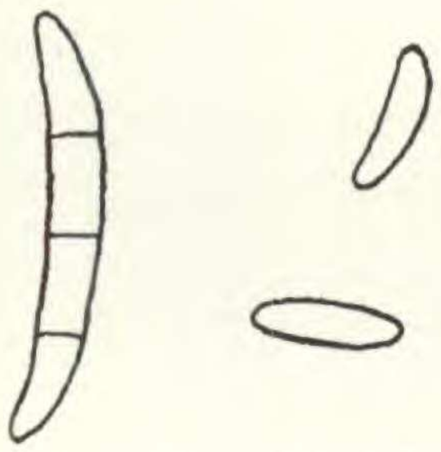


Fig. 12. Conidia obtained from a second stem of over-wintered cabbage. Camera lucida sketch × 800.

That the fungus may live over in the soil was first shown by E. F. Smith ('99, '99[a]), when he found that the organism in the soil was able to withstand drying in the laboratory for three and one-half years. Dr. M. P. Henderson in some unpublished studies on *Phoma lingam* showed inadvertently that the stumps of cabbage are not necessary for the transmission of *Fusarium conglutinans*, as it is able to live in the soil. He found that *F. conglutinans* was still present and virulent in soil that had been sifted through a fine sieve.

This experiment was repeated with soil from the experimental field at Racine. The soil was sifted through a 40-mesh sieve and in this earth cabbage was then planted. All the plants were found diseased at the end of fourteen days, while the plants in a control pot of uninfected soil remained healthy. Plants in a pot of infected soil, unsieved, showed the disease slightly earlier, at the end of twelve days, this difference in time being due probably to the fact that in the sieved soil the organism existed only as spores and, therefore, took longer to infect than where it grew rapidly from mycelium in the old host tissue.

TEMPERATURE

Literature.—Before discussing the significance of the temperature relation in the case of the attack of *F. conglutinans* on cabbage, the results obtained in other diseases where temperature has proven of pathological importance should be considered briefly for comparison.

Upon making a careful review of the literature it was found that our knowledge of this field is very limited and fragmentary, although the importance of temperature is generally recognized. Earle ('02), in a paper on the environmental factors concerned with disease, discussed the temperature relations in a general way, pointing out that for health, the plant must have temperatures of the proper degree for growth. Duggar ('09) also recognized the importance of temperature in relation to the susceptibility of the host to fungous attack, but in a paper of this nature he could make nothing more than a general statement. Reed ('10) in a similar paper paid more attention to this one of the many environmental factors involved, and showed that the temperature most favorable for the attack of a fungus is dependent entirely on the particular organism under consideration. He cited as examples of this relation the bitter rot of apple which is favored by high temperatures and the leaf curl of the peach which thrives best under cool weather conditions. Klebahn ('12, p. 88) stated that, although the temperature is undoubt-

edly an important factor in the case of diseases caused by the fungi, there has been insufficient work on the question to warrant more than a general discussion of the subject.

In spite of this lack of correlated facts on the relation of temperature to plant disease, there are many isolated notes scattered through plant pathological literature, and an attempt has been made to bring them together at this time. In order to put them in the best shape for comparison, one with another, it was thought well to arrange them according to the natural grouping of the parasites upon which the observations were made.

Schizomycetes.—The diseases caused by bacteria may be placed first. Halsted ('98) pointed out that the summer of 1894, which was excessively hot in New Jersey, was characterized by an outbreak of fire blight due to *Bacillus amylovorus.* Whetzel ('06), from evidence in New York, confirmed this relation but considered that moisture was the more important factor limiting the outbreak of an effective epidemic of this disease.

Schuster ('12), working with the bacterial rots of potato, showed that at high temperatures (35°C.) saprophytic species, as, for example, *Bacillus fluorescens,* might become parasitic on potato tubers, causing soft rot.

Smith ('14) noted that in rapidly growing shoots of susceptible hosts the incubation period of *Bacillus Solanacearum* is shortened in very hot wet weather from 8–10 days to 2–3 days. He attributes the decrease to a difference in susceptibility in the host rather than to a change in the virulence of the invading organism.

Phycomycetes.—Perhaps more work has been done on the temperature relations of the *Phycomycetes* than of any other group of fungi, but the major part of these studies has been with its relation to spore germination.

Atkinson ('95) found that high temperature was a contributing factor to damping off by *Pythium deBaryanum,* which view is corroborated by Johnson ('14) working at Wisconsin, although neither of these authors show any experimental evidence to support their opinion.

Melhus ('11) showed that chilling the conidia of *Cystopus candidus* to a temperature of 8–10°C. produced the optimum germination and also infection in the case of the radish, and thus proved that this fungus is dependent on such chilling for its best development in its attack on the host.

Similar relations have been found to hold true in the cases of other *Phycomycetes*. From field observations covering a period of twenty years Lutman ('11) concluded that *Phytophthora infestans* required a fall in temperature for its best development on the potato. Melhus ('12) showed that the optimum temperature for spore germination in this species, both conidia and zoospores, was 8–14°C., thus corroborating the previous field observations. After the fungus has entered, however, he noted in a later paper ('13) that the disease was produced more readily at comparatively high temperatures, thus showing that, in this case at least, there was a difference between the temperatures favorable to parasitic growth, depending upon whether the infecting material be spores or mycelium.

Reed ('12), working with *Phytophthora infestans* on tomato plants, found that here again its attack was dependent upon low temperatures. The attack only occurred above the altitude of 2000 feet, and then only at times when there were cool nights.

Opposed to *Phytophthora, Plasmopara Viticola* has been found to be dependent on a rather high degree of temperature. Sajó ('01) observed this in 1900 as compared with 1899, the temperature in 1900 being higher throughout the summer than in the previous year. Again, in 1912, Ravaz and Verge ('12, '12[a], '12[b]) showed that for quickest germination a temperature of 22–27° C. was necessary for this fungus and that, since the fungus found water also necessary for infection, the host could only be attacked at periods of sustained high temperatures and humidity. Istvánffi and Pálinkás ('13) showed that not only were Ravaz and Verge correct, but that the development of conidiophores and conidia from the infected host was also somewhat dependent on these same temperatures.

Ascomycetes.—In the *Ascomycetes* but little has been done on the temperature relations of the parasitic forms. In Ohio, Selby ('99, '04) observed that the leaf curl of the peach, caused by *Exoascus deformans*, was favored in its occurrence by relatively low temperatures in April, May, and June, the weather in April having the greatest influence. These conclusions were based on observations made over a period of ten years, 1893–1903. Duggar ('09) showed that the same was true in New York. Pierce ('00) found that similar conditions brought about the attack in California, and attributed the virulence of the attack to the harmful action the adverse weather had on the host, causing it to be weakened. He also noted that hot dry weather would check an attack which had already started.

That *Sclerotinia Panacis*, the cause of black rot of the ginseng root, was favored by cold weather was shown by Van Hook ('04). This author found that this disease developed only in the winter, also a time when the roots were in a dormant state.

Germination of the spores of *Sphaerotheca Humuli* has been shown by Salmon ('00) to be increased if the spores were previously exposed to cold, especially freezing, temperatures. The germination, however, took place only when the higher temperatures were restored. Sajó ('01) observed that *Oidium Tuckeri* on grapes was favored by subnormal temperature and moisture.

Probably one of the first observations of scientific value on the relation of temperature to a particular plant disease was that made on the black rot of grapes by Buchanan in 1850. As is reported by Viala ('87), Buchanan noticed that this disease was worse after a period of hot weather. Viala also made observations on this relation and found that in hot weather (maximum 35–37°C., minimum 18–20°C.) there was a bad epidemic of the trouble. When the temperature fell the disease became checked. His observations covered a period of two years. Later Edson ('03), making observations in North Carolina, came to similar conclusions.

That reaction of different parasites to the temperature relations may differ even within a single genus, is well brought out in the genus *Glomerella*. Here, on the one hand, is found *Glomerella rufomaculans* which is dependent on a maximum temperature of 32°C. for the outbreak of an epidemic (Scott, '06), while on the other, *Colletotrichum Lindemuthianum* (*Glomerella Lindemuthianum* Shear) is reported by Edgerton ('15) as being unable to grow in culture above 31°C. He shows that this species causes the most severe injury at cool temperatures, infection being inhibited by the summer heat.

Fungi Imperfecti.—Little has been done as to this relation in the *Fungi Imperfecti*. Ravn ('00) has shown that in the case of *Helminthosporium teres*, the attack on barley was conditioned on cool temperatures at the immediate time of sprouting of the kernel in the soil. Similar conditions held for the stripe disease of barley, caused by *Helminthosporium gramineum*. By growing the plants under controlled conditions of temperature, this author was able to show that a temperature of 6.5–14°C. favored the disease, while a temperature of 19–25°C. practically excluded it from the seed-beds. He showed that the susceptible period for infection was immediately at the time of germination of the seedling, and that plants sprouted in warm temperatures, which were then immediately removed to cool conditions, did not become infected. Bakke ('12), in Iowa, showed that the optimum for growth of this fungus in culture was 23–25°C., so that it would appear that the effect of the temperature was one of resistance or escape on the part of the host rather than an effect on the fungus. Further evidence bears out this belief, since *Helminthosporium teres* can cause a leaf spot in the field at the higher temperatures.

The question of the temperature relations of the parasitic species of *Fusarium* will be discussed later and may be dismissed here with a brief statement that, as a rule, they seem to require high temperatures for their most virulent attack. In this they appear to be opposed to the closely related genus, *Verticillium*, which also causes wilt diseases (Wollenweber, '13).

In one other member of the *Fungi Imperfecti, Sphaeropsis Ellisii,* Petri ('13) has observed that the attack was dependent on cool humid atmospheric conditions, and the fungus was never seen in warm well-ventilated exposures. It is probable, however, that in this disease the limiting factor is moisture rather than temperature.

Basidiomycetes.—The temperature relations of the smuts and the rusts have been worked out more exactly than the other *Basidiomycetes.* Brefeld ('95), in experiments with oat smut (*Ustilago Avenae*), showed that when germinated spores were placed in soil and oats grown therein, 27–30 per cent of the plants became infected at 15°C., while at 7°C. 40–46 per cent were attacked. Tubeuf ('01), working with the same form, found the opposite results when ungerminated spores were used instead of germinated. He showed also that the spores of *Ustilago Avenae* cannot germinate under 5°C., their minimum for germination being between 5 and 9°C. As is pointed out by Hecke ('09), the difference in the findings is probably due to the fact that Brefeld germinated the spores before exposing the cultures to the different temperatures while Tubeuf did not. On account of this difference the time of susceptibility of the host was lengthened by the low temperature in Brefeld's experiments, and hence the increased infection; while in the experiments of Tubeuf the plants at low temperatures were held below the temperature of germination of the smut spores, and, therefore, the greater infection occurred at the higher temperatures.

In regard to the stinking smut of wheat (*Ustilago Tritici*), on the other hand, the minimum temperature for germination of both the wheat kernel (3–4°C.) and the spores of the fungus (5°C.) was practically the same, while the maximum for the smut germination (25°C.) was considerably lower than that of the wheat (30–32°C.), so that in this case the opposite facts were true, as Hecke ('09) showed. Therefore, the infection was favored by low temperatures and prevented by high (25°C.), because when the plant grew slowly the length of the susceptible period was increased. Munerati ('12) reports similar observations on wheat in Italy; early fall and

late spring planting favored the host, while late fall and early spring planting increased infection.

Among the rusts a similar relation between spore germination and infection occurs. Howell ('90), working on the clover rust (*Uromyces Trifolii*), showed that infection would take place only at comparatively low temperatures, the reason given being that it was only at the low temperatures that spore germination occurred; the maximum temperature for both uredo- and aecidiospores was in the neighborhood of 25°C. Marshall Ward ('01) in his notable experiments with the brome rust (*Puccinia dispersa*) showed that the optimum temperature for germination for this form was also at 18°C.

Eriksson ('95), in making experiments with rusts, and especially with the germination of spores of different forms, found that chilling the spores in the case of *Aecidium Berberidis*, *Peridermium Strobi*, *Uredo glumarum*, and *U. coronata* accelerated germination when they were brought back to higher temperatures. Johnson ('12), working with uredospores of *Puccinia graminis*, *P. rubigo-vera*, and *P. coronata*, showed that their optimum temperatures for germination were 12–17°C.; hence epidemics of grain rusts usually spread at periods of subnormal temperatures. From these observations it is easily seen that the rusts have developed the parasitic habit to a very special degree, adapting the temperatures when there is likely to be dew as those at which spore germination will take place, and thus aiding themselves in their attack on the host.

Balls ('08) has shown in some very careful work on the temperature relation of the *Rhizoctonia* causing "sore shin" of cotton that this fungus attacks the cotton plant at 20°C., but not at 33°C. He checked his work with observations on pure cultures of the organism, and found that at high temperatures the fungus secreted, or excreted, an inhibiting substance into the culture fluid which was injurious to the fungus. Whether this same toxic substance prevented that attack on the host is questionable.

As to the wood-destroying fungi, Falck ('07) found that *Merulius silvester*, *M. domesticus*, *M. sclerotiorum*, *Polyporus*

vaporarius spumarius, and *Verpa bohemica* all have a minimum temperature for growth of about 3°C.; *Merulius silvester, M. sclerotiorum*, and *Polyporus vaporarius spumarius* have an optimum of about 25°C., while the optimum for *Merulius domesticus* and *Verpa bohemica* is at 22° C. Their maxima are all at about 30°C. It was noted that these temperatures correspond very closely to those of *Phycomyces nitens* and *Mucor Mucedo*, as determined for comparison, although each species has a rate of growth that is constant for a given temperature (other factors being equal) and characteristic of that species.

With the exception of the genus *Fusarium*, the preceding covers the important work that has been done on the temperature relation of the parasitic fungi, as far as could be ascertained. It will be readily seen that the relation of temperature to the attack of a parasite is a complex one and depends entirely upon the individual diseases under observation.

To take up now the relations of temperature to diseases caused by *Fusarium*, Jones ('08) stated in his observations on the damping off of coniferous seedlings, caused by a member of that genus, that the trouble was facilitated by high temperatures. He was confirmed in this by Gifford ('11), working on the same trouble. Wollenweber ('13), however, was the first to show this relation in the case of the wilt diseases caused by *Fusarium*. He pointed out that these diseases occur most severely in the warmer climates, especially in the tropics and subtropics, but noted the cabbage yellows as an exception to this general rule. Previously, Wolf ('10) had noted, in the case of the wilt disease of pansy (*Fusarium Violae*), that the trouble was found only in July, and then only when the beds in which the plants were growing had been heavily covered with fresh horse manure, both of which facts suggest a dependence of the fungus on high temperature. This author made no mention of temperature, nor were any experiments on this relation reported.

Orton ('13, '14) in discussing the potato plant and its relation to disease has shown that in this instance Wollenweber's hypothesis held true, the Fusarium wilt having a southern

range as compared with *Verticillium albo-atrum* which caused a trouble of almost identical nature in the northern climates. Neither of these authors made controlled experiments to determine, if possible, exact ranges of temperatures.

Humphrey ('14), working in Washington with tomato blight caused by *Fusarium orthoceras* App. and Wollenw. and *F. oxysporum* Schlecht., found that the blight was favored by high temperatures. His statements were based on observations made on experimental plots in 1911 and 1914 at Pullman, Washington, coupled with the determination of the optimum temperature for growth of the organism in the laboratory at 86°F. or 30°C. This author suggested that the light intensity and wind are also factors in bringing about the typical symptoms of the disease.

A preliminary report (Gilman, '14) of the relation of temperature to the occurrence of cabbage yellows was made at the Philadelphia meetings of the American Phytopathological Society in 1915. The full report of this work is as follows:

Field observations.—On the experimental plot at Racine during the summer of 1912 it was observed that the attack of *F. conglutinans* occurred in the early part of July, when the plants had been set about two weeks. It was noted further that the plants which escaped or withstood the disease at this time remained healthy throughout the rest of the summer. Plants set out after this period were all practically immune. Upon looking up the temperature records of the summer it was found that the attack of the disease followed very closely a period of exceptionally hot weather. Table VII gives a summary of the observations made at three different times during the growing period. The strain numbers are those used by Jones and Gilman ('15) in the development of a variety of cabbage resistant to yellows. Strains II, III, and VI were commercial varieties of Danish Ball-head imported from Denmark; strains VII, VIII, IX, and X were from seed grown from resistant heads; strain XI was of the Flat Dutch variety imported from Germany; strain XII was commercial Houser; and strain XV, commercial Danish Ball-head. Further details may be found in the publication mentioned above. The

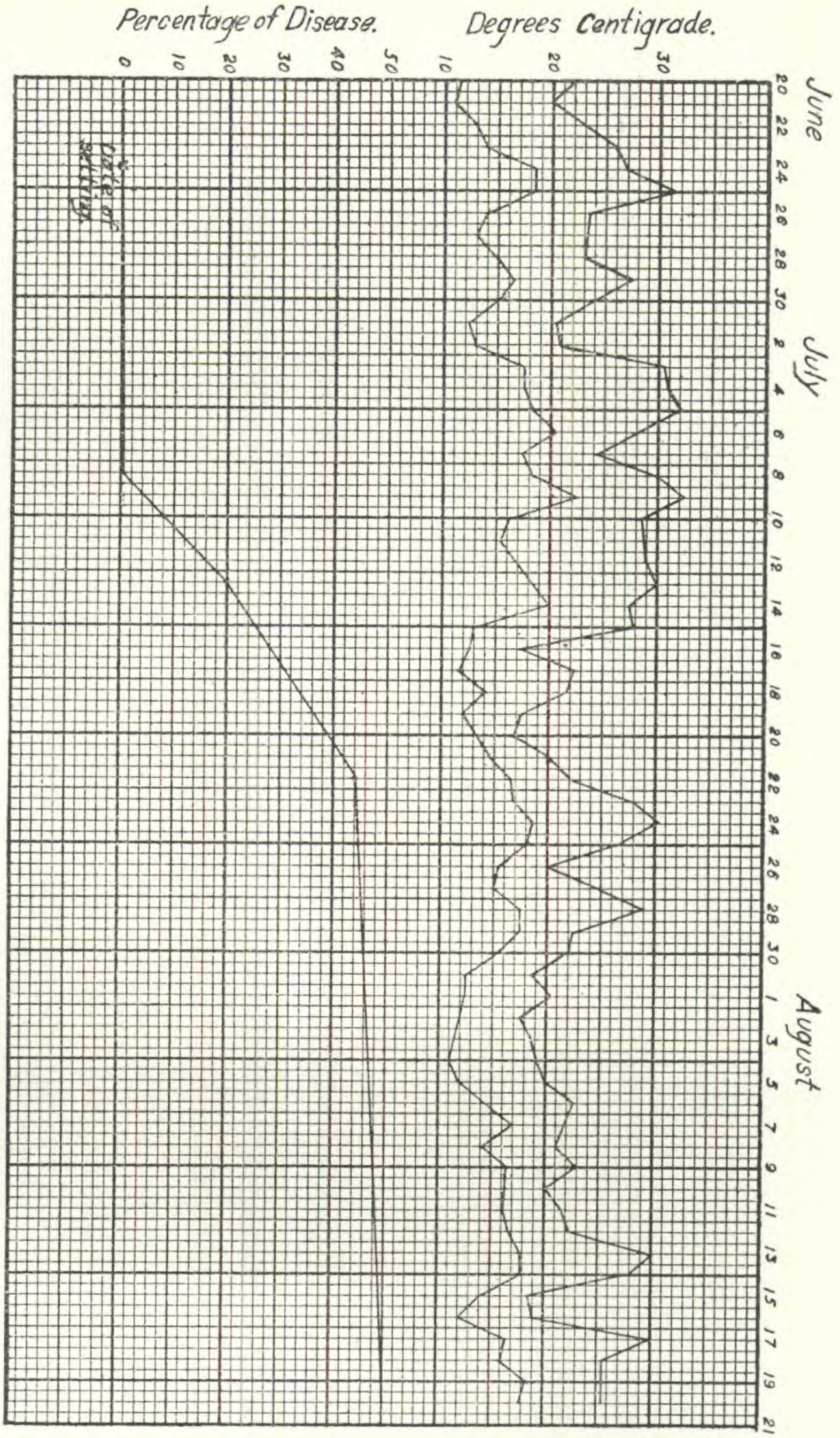


Fig. 13. Comparison of temperature with percentage of disease in field in 1912.

plants were set in the field June 24. The disease was beginning to show on July 8, when only 0.05 per cent of the plants were yellowed, and increased rapidly to 40.5 per cent on July 22. Of 420 plants set on July 15, after the hottest weather had passed, all remained healthy to August 21, but on account of the late planting did not make heads, and, therefore, were not considered later. The curves given in fig. 13 show the relation of the occurrence of the disease to temperature. The temperature data from which these were plotted are those published by the Milwaukee office of the United States Weather Bureau, a distance 25 miles north from the experimental field.

TABLE VII

SUMMARY OF FIELD OBSERVATIONS ON EXPERIMENTAL PLOT AT RACINE, WISCONSIN, 1912

Strain	Total no. of plants	July 16		July 22		August 20	
		Number yellow	Per cent yellow	Number yellow	Per cent yellow	Number yellow	Per cent yellow
II	45	18	4.0	32	71.1	37	82.2
III	45	3	6.6	24	53.3	32	71.1
VI	44	24	54.5	35	79.5	43	95.4
VII (a-y)	1039	284	27.3	551	53.0	567	54.6
VIII (a & b)	89	1	1.1	21	23.5	11	12.3
IX (3-116)	352	31	8.8	130	36.9	191	54.3
X (101-143)	625	97	15.5	195	31.2	234	37.4
XI	43	12	27.9	27	62.8	32	74.4
XII	39	5	12.8	18	46.1	17	43.6
XV	29	8	27.6	19	65.5	20	68.9
Total	2350	483	20.5	1052	40.5	1184	50.3

Again, in 1913, observations were made in the field on the same plot. The results of these observations are given in table VIII and fig. 14. Besides the strains used in 1912 several new commercial sorts were introduced. Strains XIII and XIV were Danish Ball-head bred locally, XIII being short-stemmed and XIV long-stemmed; strain XVI was Danish Ball-head grown by the Ferry Seed Company; XVII was All Season; XVIII, Succession; XIX, Volga; XX, Early Jersey Wakefield; XXI, Copenhagen Market; XXII, Early Summer; XXIII, Charleston Wakefield. While the experience of the

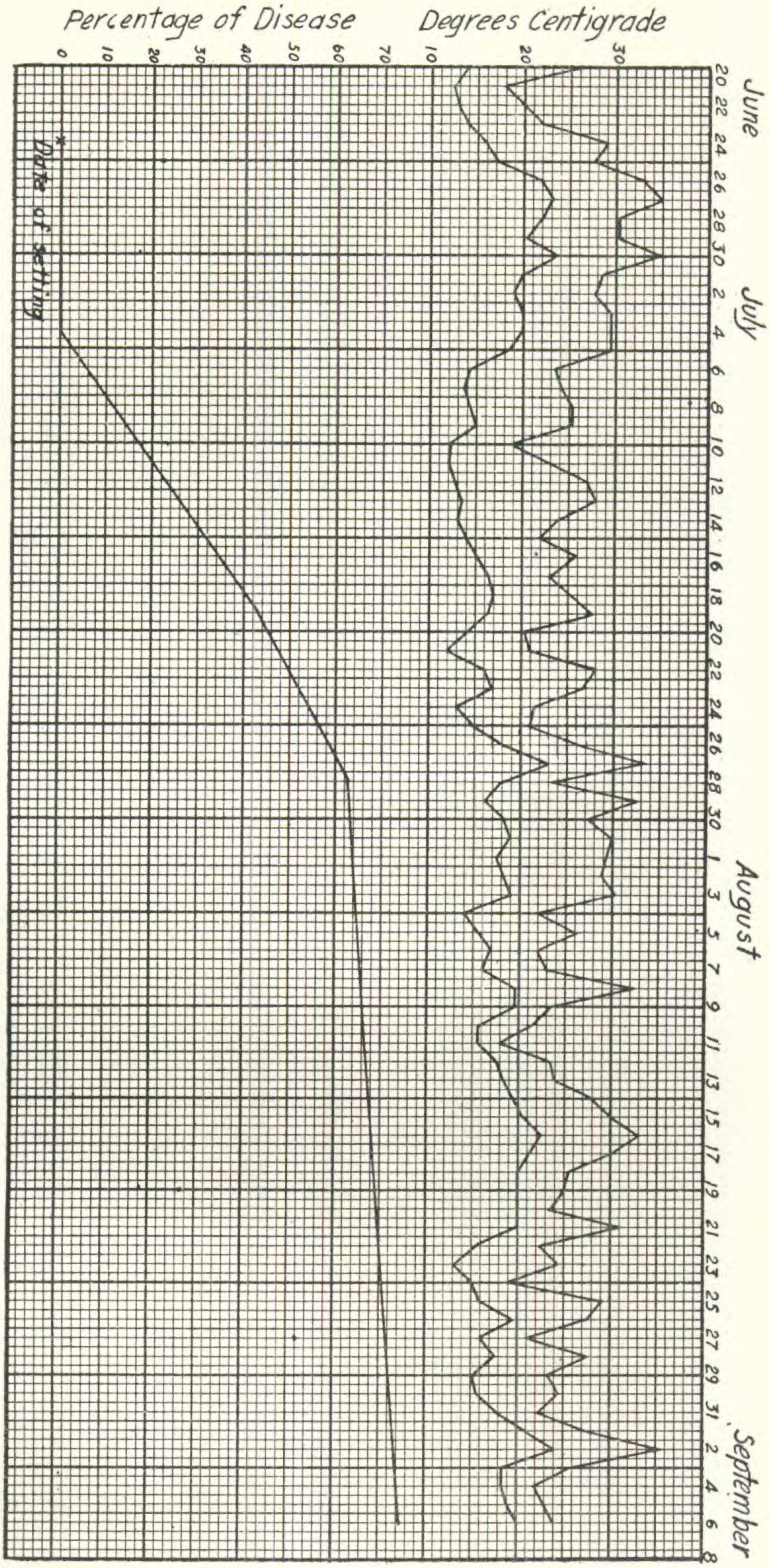


Fig. 14. Comparison of temperature with percentage of disease in field in 1913.

previous year was repeated, the relation between temperature and the attack was not as marked as it had been in 1912. The higher temperatures were sustained for a longer time, and, therefore, the percentage of disease continued to rise throughout the summer. The plants were set June 24, and no disease was found on July 4. Nevertheless, the main attack occurred in practically the same relation to the hottest weather as it had the previous year.

In 1914 the plants were grown on two experimental plots, and in addition to the strains mentioned above, a resistant strain from the Maryland Agricultural Experiment Station (XXIV) was added. The plants were set on June 26, and the disease was first observed on July 16, when 1.5 per cent of the plants showed the typical yellowing. Tables IX and X summarize the observations that were made during this summer.

TABLE VIII

SUMMARY OF FIELD OBSERVATIONS ON EXPERIMENTAL PLOT AT RACINE, WISCONSIN, 1913

Strain	Total no. of plants	July 19		July 28		September 6	
		Number yellow	Per cent yellow	Number yellow	Per cent yellow	Number yellow	Per cent yellow
II	50	28	56.0	42	84.0	47	94.0
III	46	28	60.8	34	73.9	34	73.9
VI	46	25	54.3	29	63.0	45	97.8
VII (a-y)	1243	639	51.4	874	70.3	795	63.9
VIII (a & b)	96	3	3.1	8	8.3	9	9.4
IX (3-116)	248	77	31.0	120	48.4	98	39.5
XI	47	19	40.4	21	44.7	33	70.2
XII	37	17	45.9	24	64.8	24	64.8
XIII (1-19)	820	301	36.7	489	59.6	748	91.0
XIV (1-11)	565	140	24.7	256	45.3	504	89.2
XV	985	556	56.4	707	71.7	827	83.9
XVI	43	11	25.6	25	58.1	42	97.6
XV I	40	17	42.5	26	65.0	32	80.0
XV II	28	19	67.8	20	71.4	26	92.8
XIX	42	15	35.7	17	40.5	12	28.5
XX	38	15	39.4	17	44.7	28	73.6
XXI	39	20	51.3	23	58.9	37	94.8
XXII	39	14	35.9	11	28.2	16	41.0
XXIII	33	17	51.5	17	51.5	23	69.7
Total	4485	1961	44.3	2760	51.9	3380	63.5

TABLE IX

SUMMARY OF FIELD OBSERVATIONS ON EXPERIMENTAL PLOT, HANSCHE FARM, RACINE, WISCONSIN, 1914

Strain	Total no. of plants	July 16		July 30		August 17	
		Number yellow	Per cent yellow	Number yellow	Per cent yellow	Number yellow	Per cent yellow
VIIf (1-9).....	162	3	1.85	46	28.40	54	33.33
VIIi (5-7).....	161	2	1.24	40	24.83	57	35.40
VIIIa (7-35)....	810	0	0.0	8	0.99	10	1.23
VIIIb (3-14)....	385	0	0.0	8	2.08	19	4.94
X 135.....	81	0	0.0	9	11.11	16	19.75
X 143.....	81	0	0.0	8	9.87	15	18.50
X 135 (2-33)...	782	3	0.38	145	18.54	168	21.48
X 143 (2-38)...	1213	2	0.16	215	17.72	318	26.22
XV.....	482	27	5.6	374	77.59	433	89.83
XVI.....	299	5	1.67	144	48.16	236	78.93
XII.....	81	11	13.6	67	82.7	64	79.0
XIII-11.....	81	1	1.2	69	85.2	80	98.76
XIV-8.....	81	0	0.0	62	76.5	74	91.35
XIX.....	81	1	1.2	14	17.3	19	23.45
XXIV.....	81	0	0.0	6	7.4	5	6.17
Total.....	4861	55	1.13	1215	24.78	1568	32.05

TABLE X

SUMMARY OF FIELD OBSERVATIONS ON EXPERIMENTAL PLOT, BROESCH FARM, RACINE, WISCONSIN, 1914

Strain	Total no. of plants	July 16		July 30		August 17	
		Number yellow	Per cent yellow	Number yellow	Per cent yellow	Number yellow	Per cent yellow
VIIf (5-7).....	476	7	1.47	158	33.19	262	55.0
VIIi (2-6).....	475	4	0.8	172	36.2	267	56.21
VIIIa (7-35)....	2352	0	0.0	57	2.42	176	7.48
VIIIb (3-18)....	1175	0	0.0	68	5.78	136	11.57
X 135 (8-21)...	1204	0	0.0	315	26.16	594	49.34
X 143 (2-38)...	2379	16	0.67	945	39.72	1477	62.08
XIII 11.....	238	0	0.00	175	73.5	231	97.1
XIV 8.....	236	7	2.87	170	72.0	225	95.8
XV.....	476	137	28.78	448	94.1	454	95.35
XVI.....	479	1	0.2	289	60.3	426	88.9
Total.....	9490	172	1.81	2797	29.47	4248	44.65

A soil thermograph was installed in the experimental plot; the bulb was placed six inches below the surface of the soil, and records were kept covering the growing period. These show that the temperature of the black clay loam, such as is found in Racine and Kenosha counties in Wisconsin, is comparatively high, the minimum temperature of the soil rarely falling below the minimum for the air, and the maximum temperature of the soil, because of the lag, often exceeding that of the air, especially on cold or cloudy days (fig. 15). The same relation between the main attack of the disease and temperature is apparent, although, because the high temperatures were maintained throughout July and August as they had been the previous summer, the percentage of disease also increased over a longer period than in 1912. The total percentage of the disease in this year was less than in the previous years, because plants from the resistant strains were counted with the control, the totals from the entire plot being used.

Experimental results.—The experiments to show the relation between temperature and the attack of the fungus were started in 1913. For this phase of the investigation the plants were grown in uniformly diseased soil in two different greenhouses, one of which was kept as near 25°C. as possible and the other at 15–20°C. In the first experiment three flats of infected soil and one of greenhouse soil (uninfected) were placed in the warm house, and one flat of infected soil and one of uninfected greenhouse soil were placed in the cool house. The flats were planted on October 4, 1913, with two hundred seeds in each flat. The steam was turned on November 18, at which time the plants in all the flats appeared normal in their development. On November 25, however, yellows appeared in the flats of infected soil in the warm house. The plants in uninfected soil in both houses remained healthy. Figures 16–18 give an idea of the range of temperatures in the two houses for the entire period during which these experiments were made.

On December 6 the above experiment was repeated; four flats of infected soil were planted, and two placed in each

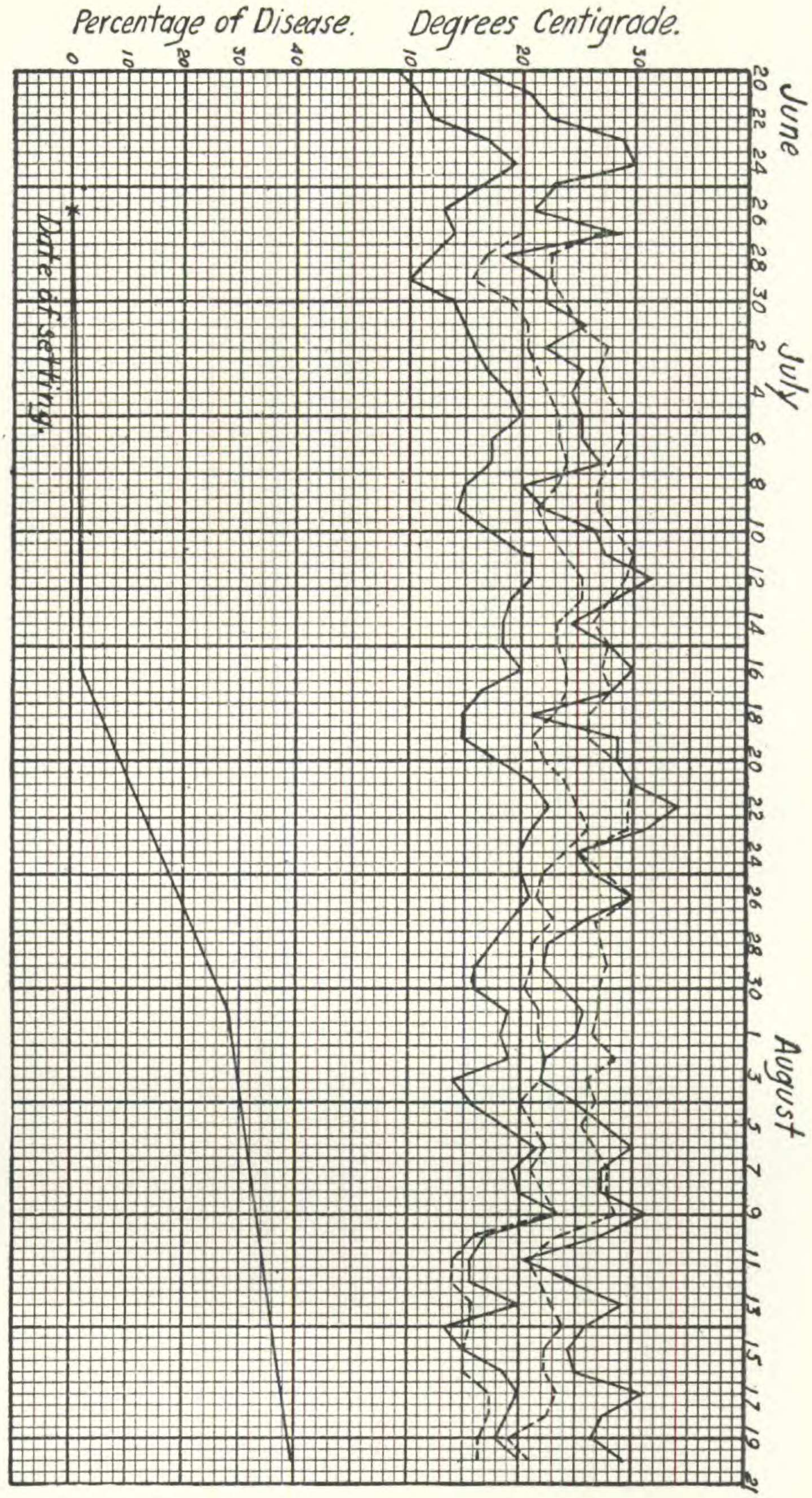


Fig. 15. Comparison of air and soil temperatures with percentage of disease in field in 1914; air temperature, solid line; soil temperature broken line.

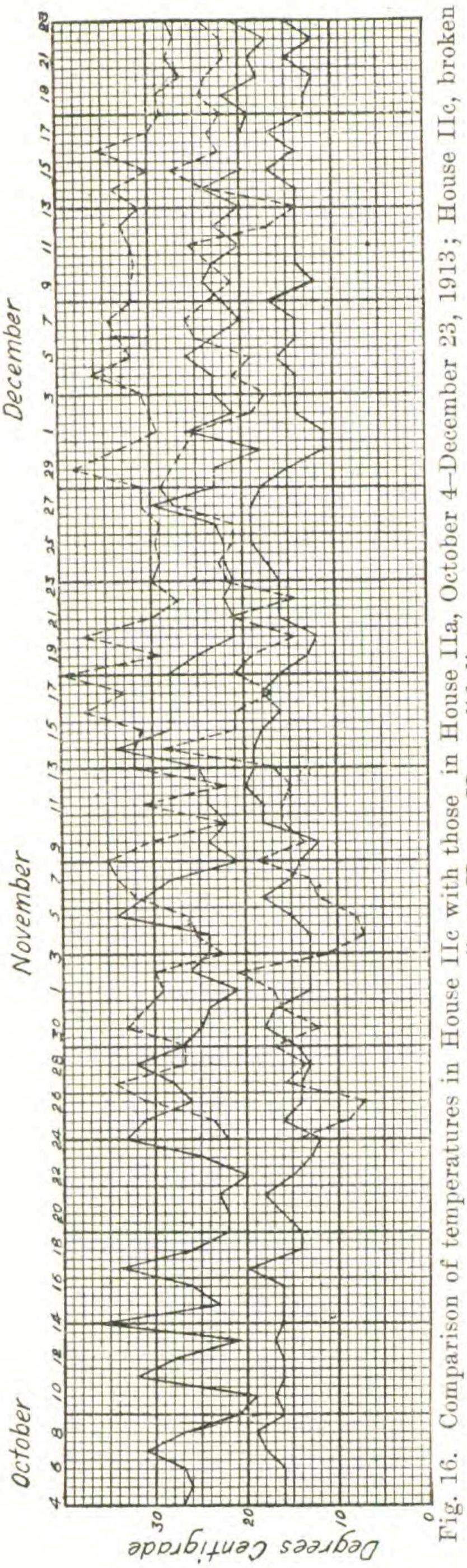


Fig. 16. Comparison of temperatures in House IIa, October 4–December 23, 1913; House IIc, broken line; House IIa, solid line.

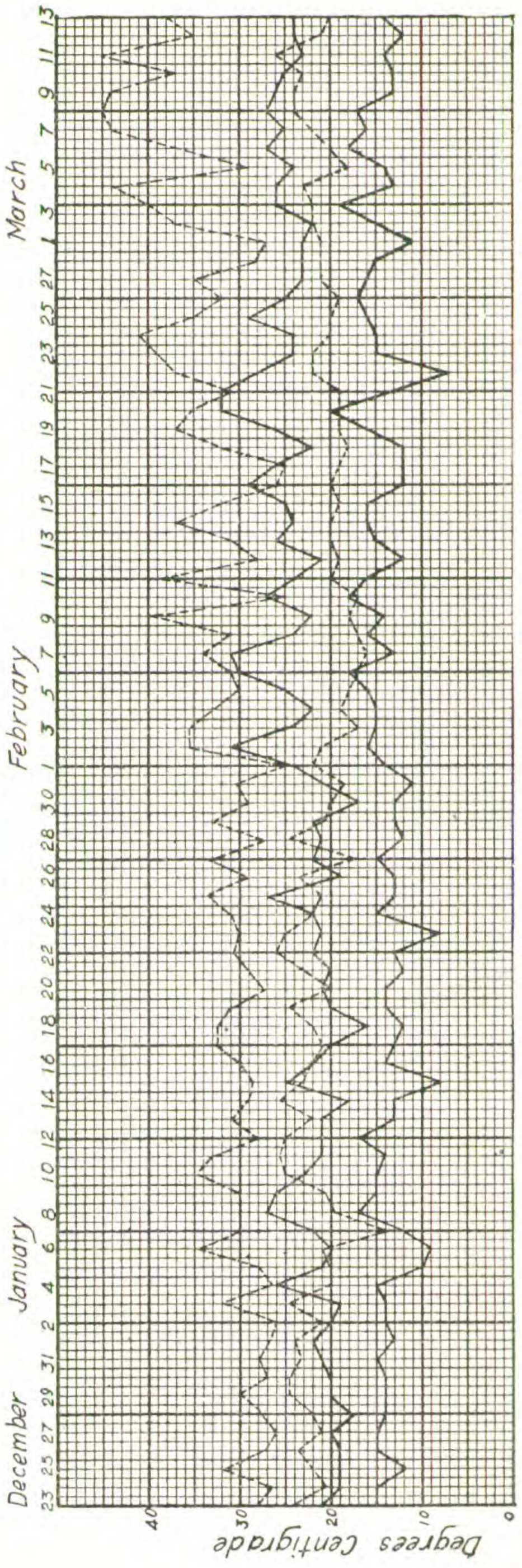


Fig. 17. Comparison of temperatures in House IIc with those in House IIa, December 23, 1913–March 13, 1914; House IIc, broken line; House IIa, solid line.

house. The yellows appeared in the warmer house on December 29. Each of the seedlings showing the symptoms of the disease was sterilized by placing the entire seedling in hydrogen peroxide and washing in sterile water. It was then placed on potato hard agar. The fungus grew readily from the stem of the infected seedlings, as is shown in pl. 2, figs. 8–11. The flats of infected soil were interchanged, and after replanting on January 29, the results were found to be the same; that is, the plants in the warmer house showed the disease, while those in the cooler house remained healthy. The first disease symptoms were observed on February 10.

During the above experiments the temperatures were as constant as they could be made in a greenhouse where the steam supply was regulated by means of an automatic thermoregulator. Of course, the heat on very sunny days was much greater than desired, but this factor could not be controlled, as shading caused too rapid elongation of the plants and a consequent susceptibility to damping off. It was found, however, that the soil temperatures were fairly constant, being from 23 to 26°C. in the warmer house and from 12 to 16°C. in the cooler house. These determinations were made directly by placing the thermometer bulb two inches below the surface of the soil and after the mercury had come to rest making the reading.

In one experiment the number of plants used in the trial was noted, and the percentage diseased after an exposure of three weeks calculated from actual count. The experiment was started January 29, 1914. The trial consisted of seven pots of infected soil, and two pots of normal greenhouse soil for controls. Five pots of infected soil were placed in the warmer greenhouse and two in the cooler house. One pot of the normal greenhouse soil was placed in each house. The disease was found first on February 10, and the plants were pulled and counts made on February 21. If the experiment had been continued, doubtless all the plants in the warmer house would have been destroyed, as they had been in the other experiments. Table XI gives the results.

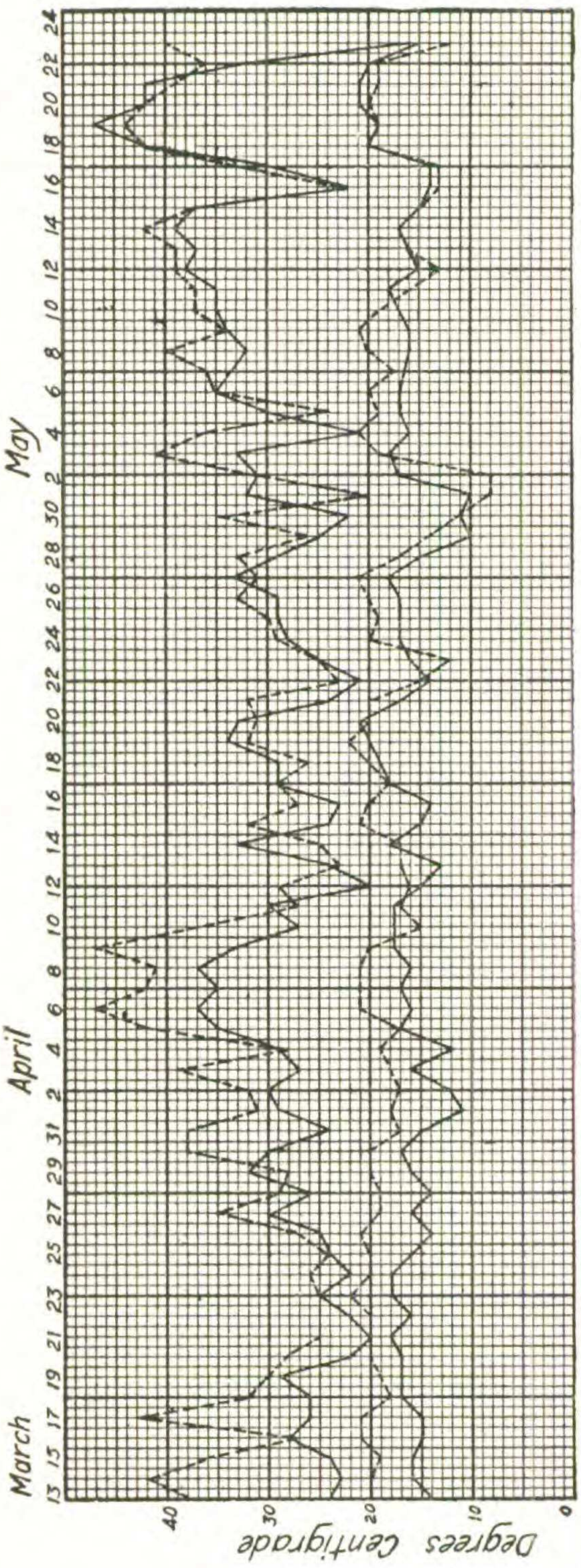


Fig. 18. Comparison of temperatures in House IIc with those in House IIa, March 13–May 23, 1914; House IIc, broken line; House IIa, solid line.

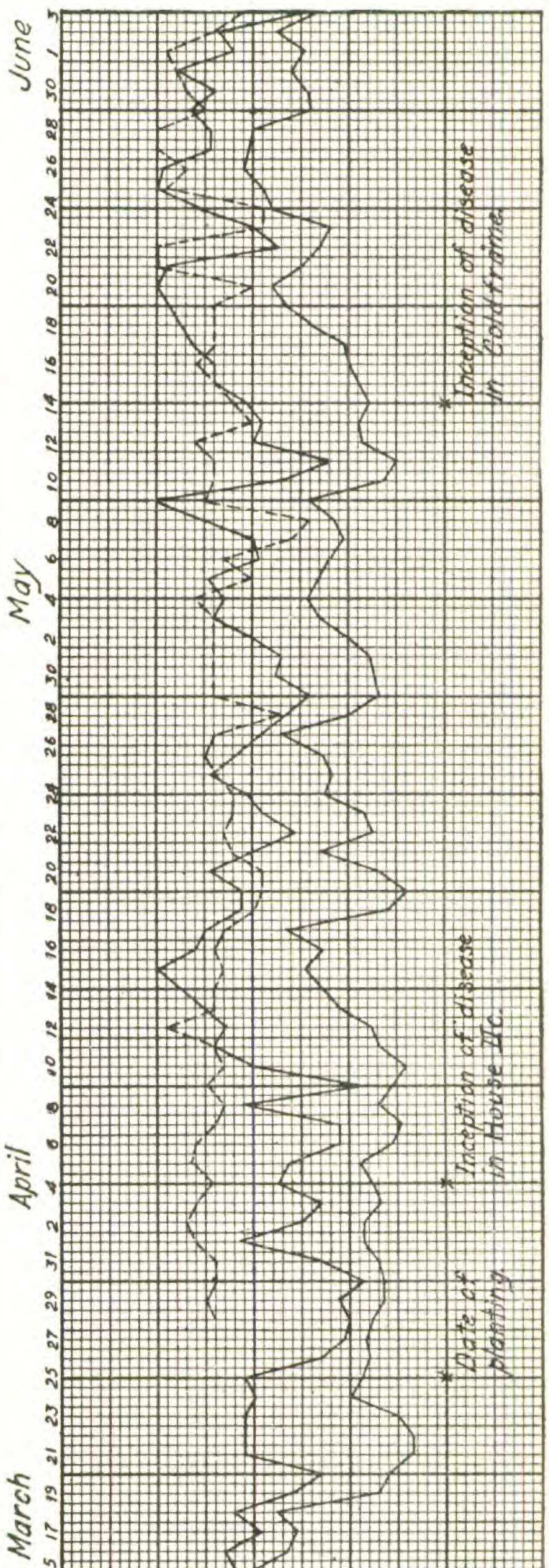


Fig. 19. Comparison of soil temperatures in the cold-frame with those in House IIc, March 15–June 3, 1914; cold-frame, solid line; House IIc, broken line.

TABLE XI

AMOUNT OF YELLOWS PRESENT AFTER AN EXPOSURE OF THREE WEEKS TO HIGH AND LOW TEMPERATURES

Pot no.	Soil	Temperature	Total no. of plants	After three weeks' exposure	
				No. diseased	Per cent diseased
1	Infected	25° C.	73	27	37
2	Infected	25° C.	69	25	38
3	Infected	25° C.	60	16	27
4	Infected	25° C.	54	20	37
5	Infected	25° C.	44	18	41
6	Infected	15–20° C.	60	0	0
7	Infected	15–20° C.	49	0	0
8	Uninfected	25° C.	119	0	0
9	Uninfected	15–20° C.	58	0	0

Again, on February 21, the effect of transplanting normal plants to infected soil at different temperatures was tried. Fifty normal plants were placed in two flats of infected soil

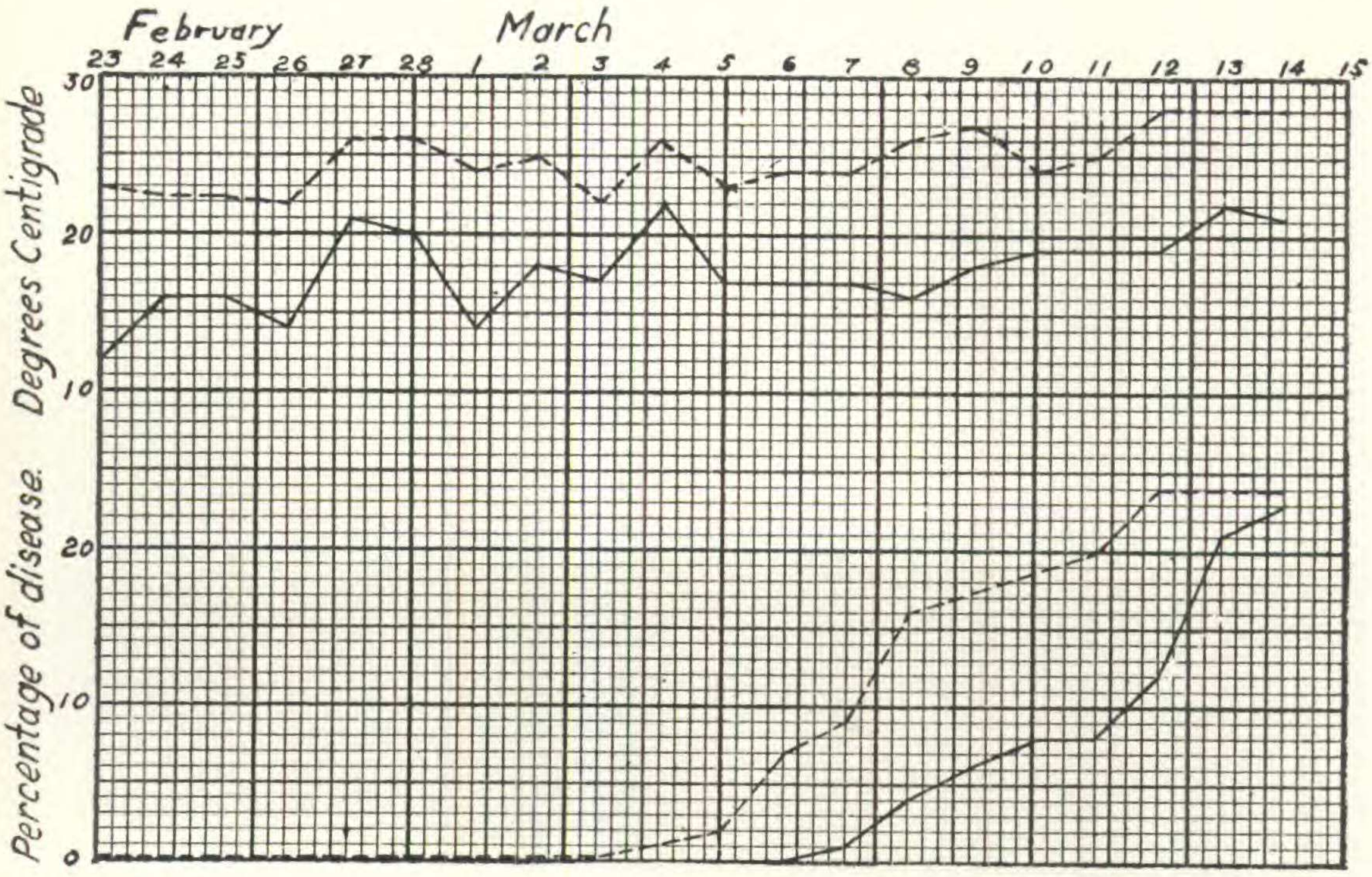


Fig. 20. Comparison of soil temperatures in House IIc and those in House IIa with the occurrence of yellows in the respective houses; House IIc, broken line; House IIa, solid line.

and one flat placed in each house. Controls consisted of ten normal plants from the same flat as the above, placed in normal greenhouse soil in two pots, one pot in each house. Soil

temperatures were taken daily. The results are not conclusive, as the temperature in the cooler house rose to 21°C. on the sixth day and remained high for two days, nor did it go back to below the temperature at which infection took place. Nevertheless, the symptoms appeared in the warmer house March 4, three days before the plants in the cool house showed any sign of the trouble, and the cooler soil retarded the advance of the fungus proportionately, as may be seen from the curves (fig. 20).

In further experiments on this temperature relation a cold-frame was used as a means of maintaining cooler conditions, for the greenhouses were all too warm, due to the increased intensity of sunlight, especially at midday. The north side of the potting-house, where the sun was excluded, made it possible to carry these cultures still further into the spring, and in all cases the results were the same.

In the experiment in which the cold-frame was used, a soil thermograph of the type manufactured by Julien P. Friez was installed. The bulb was imbedded four inches in the soil, and the temperature of the soil and air were recorded throughout the experiment. The plants were started on March 25 in six flats of uniformly infected soil, three of which were placed in the greenhouse at 25° C. and three in the cold-frame. Two pots of greenhouse soil, planted to cabbage, were used as controls in each case. The disease appeared first in the greenhouse on April 4, ten days after planting. Seedlings were plated from the diseased flats and from the flats in the cold-frame on April 9, and in all cases the diseased seedlings showed the fungus growing from the stem, while the controls remained sterile. On April 13, photographs were made of two of the flats—one from the cold-frame showing the healthy condition of the seedlings, and one from the greenhouse showing the ravages due to the attack of the fungus (pl. 2, fig. 7). The temperature records show that there was an increase in temperature with the advance of the season, and it was due to this increase in temperature that the attack occurred. The curves (fig. 19) do not show this fact well, as they are a record of the maximum and minimum only and do

not show the duration of temperature in any one day. The records themselves, while they cannot be presented here, show this increase more markedly—for the length of time of the higher temperatures increased—as the spring gave way to summer conditions. In any case the cooler condition prevented the attack of the fungus for at least a month.

In the experiment in which the flats were placed on the north side of the potting-shed, three flats of diseased soil were used. Two were placed on the north side of the potting-shed, and the third in the warm house. The flats were planted April 9, and yellows appeared in the flat in the greenhouse on April 17, while none was found, up to May 26, in the flats kept outside. Soil temperatures covering this period are shown by the curves in fig. 21.

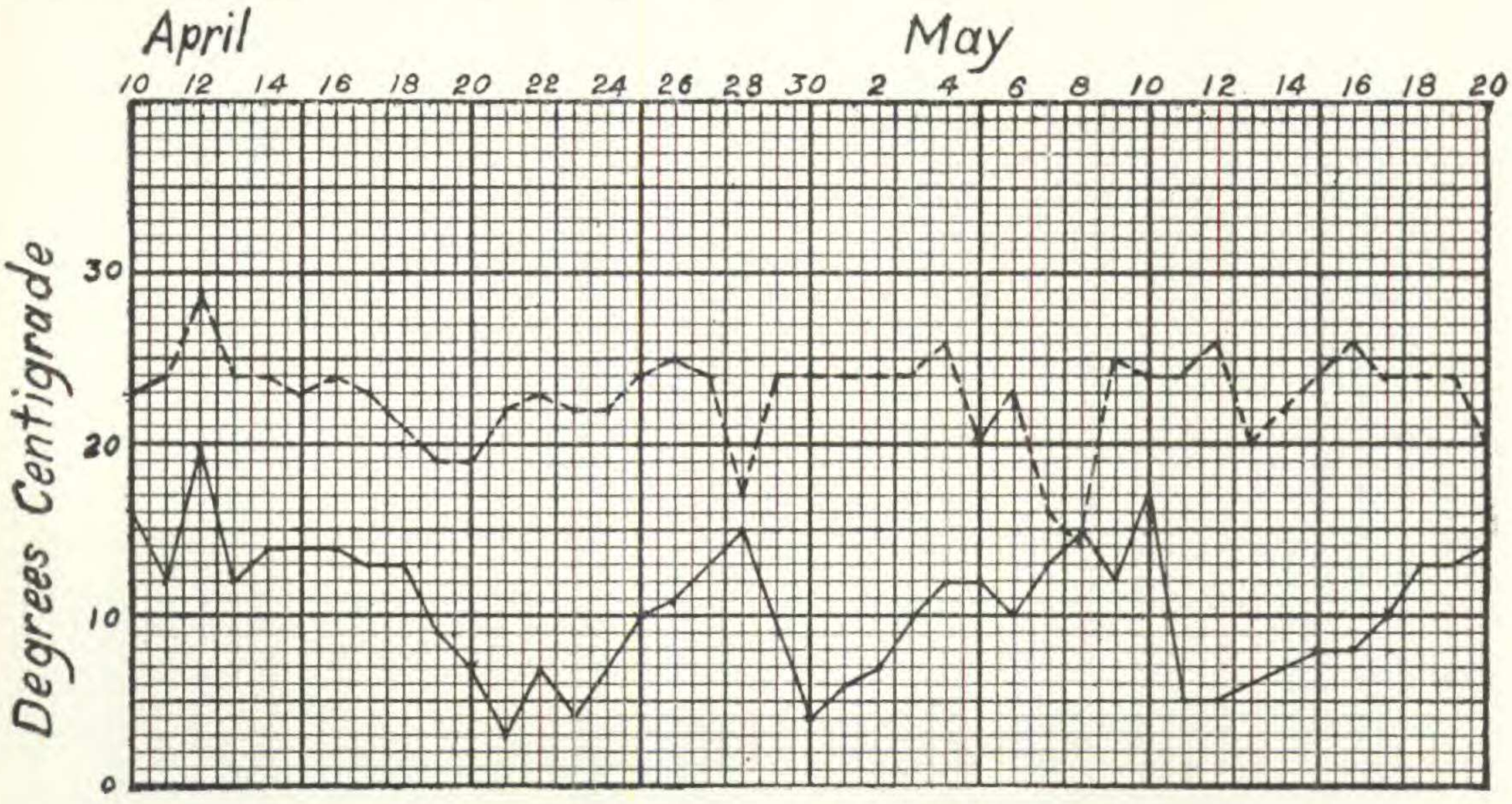


Fig. 21. Comparison of soil temperatures in House IIc with those on north side of potting-shed, April 10–May 20, 1914; House IIc, broken line; north side of potting-shed, solid line.

Further work was undertaken at the Missouri Botanical Garden to try to determine the lowest point at which the attack may occur. To this end two glass incubators were set up, and the temperature was controlled by an electric thermoregulator, so that it varied but a degree or two at the most. One incubator was set up in the laboratory at a west window, and the other was placed on the north side of the building. The temperature of the incubator in the laboratory was kept

at 22–24°C., this being the lowest constant temperature that could be procured in the room. The incubator outside was set at 16°C., but on account of the wide variation in atmospheric temperatures it was not possible to keep it constant at this point. At times the temperatures reached as high as 21°C., and at other times as low as 10°C. In spite of this variation no yellows occurred in the incubator which was outside until some days after all the plants in the warmer incubator had been attacked. Twenty pots of soil from the experimental field at Racine were placed in the warm incubator and twenty similar pots were placed in the cool incubator. The pots were put in the incubators on November 5, 1914, and in four days the plants in one pot showed wilting. The plants were sixteen days old when they were submitted to the trial. Previous to this time they had been growing in the greenhouse which was kept at 12–14°C. On November 11 yellowing was apparent in all the pots except the controls, some of the plants also showing wilting. In the outside incubator but two plants showed a slight yellowing and there was no wilting. Damping off due to *Rhizoctonia* was rather extensive in some of the pots because of the relatively high humidity conditions. Counts were taken on November 14 of the plants in the warm incubator, and seedlings from both incubators were plated on potato hard agar. The results were as follows:

At 22–24°C. *F. conglutinans* was isolated from plants from every pot of the infected soil, while none could be isolated from the plants in the normal greenhouse soil. When the plants from eight pots of infected soil, growing at 10–16°C., were plated *F. conglutinans* was isolated from but one; the fungus was not isolated from the controls. In the twenty pots at 22–24° C. there was a total of 104 plants, seven of which remained healthy, while in the incubator at 10–16° C. there was a total of eighty-eight plants, only one of which showed the disease even after they had been left until December 1 in the incubator. Table XII summarizes the results obtained on this temperature relation.

TABLE XII

SUMMARY OF RESULTS OF EXPERIMENTS TO SHOW THE RELATION OF TEMPERATURE TO ATTACK OF THE FUNGUS

Incubation period in days	Extent of trial: Infected soil, Higher temperature	Extent of trial: Infected soil, Lower temperature	Extent of trial: Uninfected soil, Higher temperature	Extent of trial: Uninfected soil, Lower temperature	Approximate average temperature in °C.: Higher	Approximate average temperature in °C.: Lower	Condition of control at inception of disease
13	3 flats.	1 flat..	1 flat..	1 flat..	22°	16–20°	Healthy..
21	1 flat..	1 flat..	1 pot..	1 pot..	22°	16–20°	Healthy*.
30	10 pots.	12 pots.	1 pot..	1 pot..	22°	14–16°	Healthy..
4	20 pots.	20 pots.	3 pots.	3 pots.	22–24°	10–16°	Healthy*.
23	3 flats.	1 flat..	1 flat..	1 flat..	25°	14–18°	Healthy..
12	3 flats.	1 flat..	1 flat..	1 flat..	25°	14–18°	Healthy..
12	7 pots.	5 pots.	2 pots.	2 pots.	25°	14–18°	Healthy..
8	2 flats.	1 flat..	2 pots.	2 pots.	25°	14–16°	Healthy..
10	3 flats.	3 flats.	2 pots.	2 pots.	30°	14–16°	Healthy*.

*In these cases the controls in infected soil at the lower temperatures became diseased later, due to a rise of temperature above the point at which they were able to resist the disease.

In a further experiment seeds were planted on October 20 in pots of infected soil and allowed to stay in the greenhouse. The soil temperature was 10–16°C., and while a little disease appeared from time to time after December 1, 1914, the attack was very light and very few of the plants suffered. When on February 2, five pots of these plants were taken to a greenhouse whose air temperature was 28–30°C., yellows appeared in virulent form in three days and all but three plants were dead on February 20. Plate 1, fig. 3, shows typical pots from this experiment. It was repeated February 28 with similar results.

The fact that high temperatures caused the yellowing of the cabbage when the plant was attacked by *F. conglutinans* having been clearly established, the next point was to find, if possible, whether the fungus entered the host at the lower temperature or not. The first experiments were made by plating from the plants in the lower temperatures, especially from parts of the roots of plants grown in infected soil. Although the plates were made in the same manner and with the same

care as in the case of the yellowed seedlings grown at higher temperatures, at no time was *F. conglutinans* isolated from the roots of these plants. Controls from roots grown at the higher temperatures showed the fungus, as has been pointed out in previous experiments. Experiments were therefore instituted to test whether any such relation might be indicated by indirect methods.

The first experiment was started on April 21, 1914, at which time twenty pots of infected soil were planted to cabbage, and all were placed on the north side of the pottingshed where a low temperature could be maintained. They were kept here until June 6, sixteen days, when all but two were placed in House IIc which was being kept at approximately 25°C. On June 9, after the pots had remained in the warm house for three days, pairs of pots were removed to the cooler temperature at intervals of two days until June 15, after which date a pair was removed each day until June 19. On June 30 yellows appeared in the two pots removed on the last day, June 19, but none was found in any of the other pots. This experiment showed that in this case the yellows appeared in the same length of time as it usually took to appear in a warm house, and would lead to the opinion that there had been no infection at the low temperature, or if the plants had been attacked, that they were able to recover under favorable conditions for growth.

Coincident with this last experiment, moreover, twenty pots of infected soil planted to cabbage were placed in House IIc, and each day two pots were removed to the cooler temperature. No yellows appeared in any pots removed in the first eight days, but in all those removed subsequently yellowing was found on June 2. Controls in the warm house showed the first symptoms on June 1, one day earlier than those on the outside. The cooler temperature, therefore, checked the disease in most cases, but where it had gone too far, the only effect was a slight lengthening of the period of incubation.

Later observations on this point do not seem to confirm these results. It will be noted that in the experiments carried on at the Missouri Botanical Garden, when the plants

were first grown in the greenhouse and then placed in the incubator at 22–24°C., the disease appeared in but four days, a period that was shorter than had been noted in any other experiment. This trial was repeated on March 9, and again the seedlings showed the disease in four days, on March 13. Eight pots of seedlings were used, and the disease appeared in all the pots on the same day, although not all the seedlings in any one pot were yellow at this time. Previous to the appearance of the yellows, platings made from the roots by the hydrogen-peroxide method gave negative results in all cases. Further, the roots were washed out of the soil and examined carefully under the microscope, but no hyphae of the fungus were observed until after wilting or yellowing had begun. The rotting usually began at the tips of roots near the surface of the soil, and progressed toward the main roots and stem. The only explanation that seems applicable to these conflicting results is that, because the temperature in the greenhouse at the Garden is slightly higher than that found on the outside of the potting-shed, the fungus may enter to a limited extent, but cannot affect the host unfavorably except at the higher temperature, while at Madison it was unable to gain any sort of a foothold. This view is further supported by the fact that a few plants grown in diseased soil in this greenhouse, after a long period of time showed yellows, as previously mentioned.

Because the small number of hyphae found in any single diseased stem seemed insufficient for the blocking of the passage of water to the leaves of the diseased plant, some preliminary work was undertaken to find, if possible, whether mechanical or chemical killing of the stem might bring about symptoms in the leaves similar to those produced by the fungus, and especially with regard to the production of a toxic substance to which the symptoms might be ascribed.

To test this question six plants of cabbage were cut on one side with a scalpel so that half of the stem was removed for a distance of 0.5 cm. The plants were about two weeks old and growing rapidly. The cut surfaces were covered with paraffin to prevent too rapid drying of the tender tissues. Two

plants showed wilting in nine days but the others all remained upright and turgid, having completely recovered. There was no discoloration of the leaves in connection with the wilting.

In a later experiment with older plants, the entire stem of each plant was killed for a distance of 3 cm. from the surface of the ground by allowing it to stand in alcohol for three minutes. After nine days, wilting appeared in one of the five plants, the lowest leaves drooping first, but with no discoloration such as occurs under the influence of fungous attack, nor falling of the wilted leaves. A second plant succumbed on the twelfth day, but again there was rapid wilting with no loss of green coloring matter. By the eighteenth day all the plants had wilted, but even where the injury had been least and the wilting slowest there was no discoloration or falling of the leaves. The experiment was repeated with older plants in March, 1915, with similar results. The wilting always took place without discoloration of the leaves, nor did any of them drop before the entire head was wilted.

Further work was started, therefore, to see whether the fungus could produce in pure culture any substances toxic to cabbage. For this study two Erlenmeyer flasks of half-liter capacity, each containing 100 cc. of Uschinsky's fluid, were inoculated with a virulent culture of *F. conglutinans*. After two weeks the fungus-felt was filtered from the solution by means of a pressure filter, and the solution, after dilution to 500 cc., was poured in two glass tumblers, in which germinated cabbage seedlings were then placed. Controls of Uschinsky's fluid diluted 2:5, tap water, and Pfeffer's full nutrient were used in connection with the experiment. Difficulty was experienced in getting the plants to start because of the desiccation of the young cotyledons. By placing the plants in an incubator under humid conditions, the plants growing on tap water and Pfeffer's solution grew fairly well, but on Uschinsky's fluid, neither on that in which the fungus had been growing nor on the sterile fluid, was it possible to get any growth, indicating that some other media or methods will have to be used. Further work on this point is being pursued.

Discussion.—Exactly why the raising of the temperature should bring on this disease is still not clear, but in view of our present knowledge some correlation should be made between the relations found and the other work that may shed light on this point. First, it should be pointed out that many of the so-called vascular parasites behave in a very similar manner toward temperature. As Smith ('14) has shown with *Bacillus Solanacearum*, Humphrey ('14) with *Fusarium orthoceras*, and the present investigation with *F. conglutinans*, high temperatures facilitate the destruction of the host. To what extent this destructiveness may be attributed to changes in the parasite and to what extent to changes in the host plant, it is difficult to determine. Smith and Humphrey both are inclined to consider the changes in the host the primary factors concerned, and as will be pointed out, the same opinion may be taken in the case of the cabbage disease. Nevertheless, the change in the fungus must be looked into also.

Among diseases of plants that are partially dependent on temperature relations for their occurrence, in many of the cases the relation is not one of loss of virulence on the part of the fungus but a limitation in the temperature range of germination of the fungous spores. This sort of limitation was best illustrated by the work of Melhus ('12, '13) on *Phytophthora infestans* as related to the potato blight. This author showed that, although the spores germinated only at low temperatures, the mycelium which wintered over in the tuber, attacked the new shoots from such tubers only at high temperatures. Other cases of similar nature, where the temperature for spore germination differed from that of mycelial growth, are found among many of the obligate plant parasites. Examples that might be cited are *Cystopus candidus, Plasmopara Viticola, Ustilago Avenae, U. Tritici, Uromyces Trifolii, Peridermium Strobi, Puccinia graminis, P. rubigo-vera, P. dispersa,* and *P. coronata.*

That *Fusarium conglutinans* is not dependent on germination temperatures for its destructive attack is clearly shown by the fact that germination occurs readily at 17°C., which temperature is close to the lower limits of its destructiveness

in the case of cabbage. Moreover, it grows readily at temperatures much lower than this. The raising of the temperature merely increases the rapidity of the growth of the fungus and, therefore, as far as this investigation is concerned, the high temperature from the standpoint of the fungus aids its destructiveness by increased spread in the soil, and more rapid development in the vascular system after it has once entered. Other possible relations, such as that of production of toxic substances, remain to be worked out.

From the host standpoint the effect of temperature is much more complicated. Appel ('15) in his discussion of leaf roll in potato considered excessive transpiration of prime importance in bringing about this condition whether the cause of the trouble was parasitic or not. The symptoms of the disease in the cabbage indicate that the phenomena involved are very similar to those concerned with the annual autumn fall of leaves from woody plants. The discoloration (yellowing in the diseased plants), the formation of an abscission layer, and finally the fall of the leaves are in all ways comparable. Hence the same physiological changes within the plant are probably taking place. From this point of view then, the work of Molisch ('86) and Varga ('11) on the relations of environmental factors to the fall of leaves gives a basis for an explanation of the symptoms from the host standpoint. Molisch showed that a slow but continued decrease of water content of the fundamental tissue of the leaf led to the formation of an abscission layer and finally fall of the leaf. He further found that this loss of water might be brought about by increased transpiration or by decreased absorption or conduction from the roots to the leaf. Temperature influenced leaf-fall, both indirectly through its effect on transpiration, and directly by bringing about the formation of the abscission layer. Leaves fell at 17–22°C. more rapidly than at 1–10°C. when other conditions were equal. Varga studied the relation of temperature to leaf-fall more exactly and found that, as a rule, low temperatures lowered transpiration and thereby set up a stimulus to leaf-fall, but that if the abscission layer had been formed through other influences, higher tempera-

tures within limits, caused a more rapid fall of the leaves. These facts give a possible explanation of the results found with cabbage yellows. The hyphae of *F. conglutinans* in the fibro-vascular bundles cause a constant but slow drain on the water content of the plant, which causes the formation or the beginning, at least, of the formation, of the abscission layer. High temperatures, in addition to causing increased growth of the fungus, raise the transpiration and also stimulate leaf-fall; thus all the factors are cumulative in their effect.

The reason that mechanical and chemical injuries to the stem did not cause similar symptoms may be explained by the fact that the plants wilted before sufficient time was given for the formation of the abscission layer and, therefore, the difference in symptoms. This theory also concurs with that of Humphrey in regard to the tomato blight, but a large amount of work is still necessary before it will be completely proven.

Summary

Cabbage yellows is a wilt disease of cabbage caused by *Fusarium conglutinans* Wollenw.

The fungus is a facultative parasite living in the soil, from which, under certain conditions, it becomes destructive to cabbage.

The fungus has a high optimum temperature and is very resistant to drying—both in pure culture and in the soil.

Inoculation experiments with *Fusarium conglutinans* in pure culture caused the disease in a large percentage of the trials. Control plants remained entirely free from the yellows. *Fusarium conglutinans* was recovered from inoculated diseased seedlings and again produced the disease upon inoculation.

Variation in virulence of the cultures and in susceptibility of the host caused many artificial inoculations to be unsuccessful, resulting in less than 100 per cent infection.

Mechanical or chemical injury to the stem of the host caused wilting, but neither yellowing nor dropping of the leaves such as is found in diseased seedlings.

The characteristic symptoms are dependent on a temperature of about 17–22°C. or above for their occurrence. Lower temperatures (12–16°C.) under controlled conditions prevented the occurrence of the trouble in the greenhouse.

Observations made in the field during the summers of 1912, 1913, and 1914 bore out this relation between the occurrence of the disease and high temperature.

In conclusion, the writer wishes to express to Dr. L. R. Jones, at whose suggestion this investigation was undertaken, and to Dr. B. M. Duggar, under whom it was completed, his sincere appreciation of the many valuable suggestions and helpful criticisms given during the progress of this work. He is further indebted to the support of the Wisconsin Experiment Station for the opportunity of conducting the initial stages of the work and to the Missouri Botanical Garden for the completion of the work upon the problem.

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EXPLANATION OF PLATE

PLATE 1

Fig. 1. Results of inoculation in the greenhouse with pure cultures.
Pot No. 1. Infected soil, sterilized.
Pot No. 2. Sterilized soil inoculated with pure culture of *F. conglutinans.*
Pot No. 3. Soil from infected field, untreated.
Madison, Wisconsin, July 14, 1913.

Fig. 2. Results of inoculation in the garden with pure cultures.
Pot No. 1. Infected soil, sterilized.
Pot No. 2. Sterilized soil inoculated with pure culture of *F. conglutinans.*
Pot No. 3. Soil from infected field, untreated.
Madison, Wisconsin, July 14, 1913.

Fig. 3. Effect of temperature on the attack of *F. conglutinans* on cabbage.
Pot No. 1. Uninfected soil in cool house.
Pot No. 2. Infected soil in cool house.
Pot No. 3. Infected soil in warm house.
Pot No. 4. Uninfected soil in warm house.
Missouri Botanical Garden, March 1, 1915.

Fig. 4. Diseased cabbage plant showing typical one-sided bending of leaf and loss of lower leaves. Madison, Wisconsin.

Fig. 3

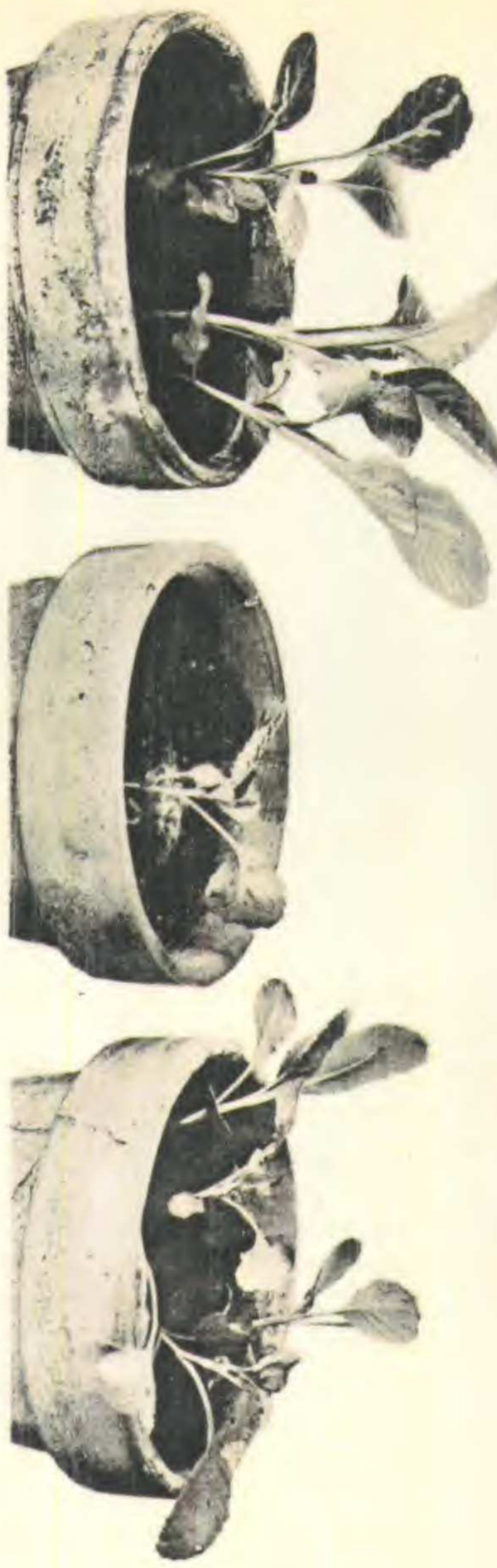

Fig. 2

Fig. 1

Fig. 4

GILMAN—CABBAGE YELLOWS

Explanation of Plate

PLATE 2

Figs. 5 and 6. Comparison of rate of germination of resistant and commercial varieties of cabbage under the same conditions. Fig. 5, commercial sort; fig. 6, resistant. Missouri Botanical Garden, November 5, 1914.

Fig. 7. Effect of temperature on the attack of *F. conglutinans* on cabbage. On left, flat from cold-frame; on right, flat from House IIc. Madison, Wisconsin, April 13, 1914.

Figs. 8, 9, 10, and 11. Effect of temperature on the attack of *F. conglutinans* on cabbage. Fig. 8, plants from temperature control in cold-frame; fig. 9, plants from soil control in House IIc; fig. 10, plants from Flat No. 1 in House IIc; fig. 11, plants from Flat No. II in House IIc. Flats Nos. I and II and the temperature control all contained infected soil. Soil control was uninfected greenhouse soil. Madison, Wisconsin, April 11, 1914.

Fig. 12. Branched cabbage plant, one branch, *DE*, showing yellows, while other, *BC*, remains healthy. Platings made from marked points and results shown in fig. 13. Madison, Wisconsin, May 19, 1914.

Fig. 13. Plate made from plant shown in fig. 12. Note that pieces *B* and *C* from healthy branch remained sterile. Madison, Wisconsin, May 21, 1914.

Fig. 14. Stems of infected cabbage plant on potato hard agar. Note mycelial growth from vascular bundles and ends of cut stem. Madison, Wisconsin.

Figs. 15 and 16. Comparison of results of inoculation experiment. Fig. 15 shows three pieces of stem from each of three healthy cabbage plants grown in sterilized soil; fig. 16 shows the same from three diseased plants grown in soil that had been sterilized and inoculated with pure culture of *F. conglutinans*. Madison, Wisconsin, July 16, 1913.